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QUADERNI DI COOPERAZIONE SANITARIA

HANDBOOK OF MALARIA INFECTION IN THE TROPICS

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INTRODUCTION

Malaria is probably the second infectious cause, after tuberculosis, of morbidity and mortality all over the world. It has been estimated that 300-500 millions new clinical cases of malaria (more than 90% of which in tropical Africa) occur every year worldwide, causing 1.5 to 2.7 millions deaths, the great majority being recorded in African children (World Health Organization, 1994)[1]. In the age-group 1-4 years, malaria is the cause of nearly one-quarter of all deaths in some african Countries. Outside Africa, other Countries pays a heavy tribute to malaria. Among them India, Brazil, Sri Lanka and Afghanistan are the most involved.

The introduction of antimalarial specific drugs and of residual insecticides during the '50s caused the disappearance of malaria transmission or at least the decrease of malaria-specific morbidity and mortality in several Countries, but has mislead the scientific community that world wide eradication of the disease was to be achieved soon.

As a matter of fact, the number of malarious Countries decreased from 140 in 1955 to 90 in 1992 but these preliminary achievements were not doomed to be followed by the complete success of mankind in the everlasting struggle against this dreadful disease. Furthermore, from the other side, they caused a sharp fall in the interest (and in the amount of dedicated funds for research!) of the scientific community in a disease that was thought to be the second infectious plague to be dominated by man after smallpox.

Unfortunately, logistic, economic and political reasons have impeded substantial progresses in those areas, as Africa, that would have benefited more from malaria eradication activities. Too late it was realized that the eradication of malaria from a given area needs the simultaneous development of the health, agricultural, sanitation and economic systems, that were not to be achieved in the last 30 years in a large number of malarious Developing Countries. It is not by chance, in fact, that eradication activities were successful in the more economically advanced temperate and subtropical areas of the world. The worldwide spread of drug-resistant *Plasmodium falciparum* strains, that has interested virtually each continent except Central America, and the initial resistance of some mosquitoes to residual insecticides have further hampered the success of malaria eradication campaigns.

Having realized the impossibility to achieve the eradication target untill conditions for a more global development of involved areas were achieved, the scientific community has then addressed the issue of control programmes.

In 1992, out of 5.430 million people inhabiting the planet, the following figures reflect the world-wide situation of malaria:

3.150 millions people live in malaria free Countries;

1.780 millions people live in areas were malaria was reduced or even eliminated but it was reinstated;

500 millions people live in areas (mainly tropical Africa) where malaria situation remains unchanged despite control activities.

It has also to be remembered that the increasing volume of international travels and migration flow to and from malaria endemic areas has also exacerbated the problem of imported malaria in otherwise malaria-free areas, where peculiar ways of transmission have been reported (airport malaria, transfusion malaria, malaria in drug addicts). Imported infected mosquitoes have proved capable of transmitting the infection even far from International Airports (*baggage malaria*) so that the ghost of reintroduction of malaria infection (mainly *Plasmodium vivax*) in subtropical and temperate malaria-free areas has already been evoked (World Health Organization, 1995)[2].

It is nevertheless in Developing Countries of tropical areas, and particularly in the most peripheral levels of the health systems, that malaria still represents a major health problem that is to be addressed by local health systems with the support of the international community.

The mainstones of malaria therapy and diagnosis have not substantially changed over the last fifty years (the only possible exception being represented by the introduction of the new drugs mefloquine and halofantrine) and new effective and economic drugs and diagnostic tools that could be used in the field are eagerly awaited, as well as the availability of effective malaria vaccine.

In the field of therapeutics, great expectations have been raised by the artemisinin derivatives that proved to be highly and quickly effective to clear chloroquine-resistant *P. falciparum* parasitaemia in many field trials (Supplement to the Transactions of the Royal Society of Tropical Medicine and Hygiene, 1994)[3].

In the field of diagnosis, the easy-to-perform but time consuming microscopic methods could be supported in the future by biotechnology-based methods, but still economic constraints are to be overcome.

After decades of deceiving attempts, the Spf66 vaccine appeared promising, even if the results of the subsequent trials carried out in the Gambia and Thailand failed to confirm the preliminary encouraging results (D'Alessandro *et al.*, 1995; Nosten *et al.*, 1996)[5,6].

It is then conceivable that the tools available for fight against malaria in its core areas, that are the peripheral areas of tropical endemic Countries, will change little in the next years. Health workers operating at the district level are in the front line in the fight against malaria and have to cope with economic and logistic constraints and lack of access to scientifically sound informations. This **Handbook of malaria infection in the tropics** is dedicated to them, hoping that it will become a useful tool in the bedside clinical activities, in control activities and in the understanding of the future line of research in the field. The Non Governmental Organization **Amici di Raoul Follereau** has financially supported the printing and distribution charges of this booklet, that is the 15th issue of the Health Cooperation Papers collection started in 1980 and we are pleased to testify the dedication and collaborative attitude of all their staff. **All the Authors** are of wide international reputation and have collaborated benevolently to this project with the valuable presentation of their experience. To them we would like to address our personal thanks for finding the time to give their contributions among several other engagements. Also, we would like to thank the **World Health Organization** for its permission to reproduce excellent didactic plates and figures.

The success of this Handbook will be achieved only if it will be regarded as an operating and useful tool, and not as a reference book, to help health policy makers and, in particular, health worker at the district level to fight against "mala aria" (bad air).

Prof. G. Carosi M.D., Ph. D.
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LIFE CYCLE OF MALARIA PARASITES

A. Matteelli, F. Castelli, S. Caligaris

Introduction

Plasmodia have arisen from the Coccidian stem (table 1) and share most of the typical features of the *Apicomplexa*. They probably represent an evolutionary progress in respect of ancestors (of the *Eimeriide* family) who just develop in the intestinal tract and have a single host. Plasmodia, in fact, present a sexual multiplication phase in the insect vector, a first asexual multiplication phase in the tissue of the vertebrate host, followed by the main asexual multiplication phase in the blood.

Sub-kingdom	Protozoa
Phylum	Apicomplexa
Class	Sporozoasida
Sub-class	Coccidiasina
Order	Eucoccidiorida
Sub-order	Haemospororina
Family	Plasmodidae

Table 1. Classification of human protozoa of the genus *Plasmodium*.

	P. vivax	*P. ovale*	*P. malariae*	*P. falciparum*
Pre-erythrocitic cycle (days)	8	9	13	5-6
Pre-patent period (days)	11-13	10-14	15-16	9-10
Incubation period (days)	13 (12-17)	17 (16-18)	28 (18-40)	12 (9-14)
Number of merozoites/tissue schizont	over 10,000	15,000	2,000	40,000
Hypnozoites	present	present	absent	absent
Erythrocitic cycle (hours)	48	50	72	48
Average parasitaemia/μl	20,000	9,000	6,000	20-500,000
Maximum parasitaemia/μl	50,000	30,000	20,000	2,000,000

Table 2. Some characteristics of infection with four species of human Plasmodia.

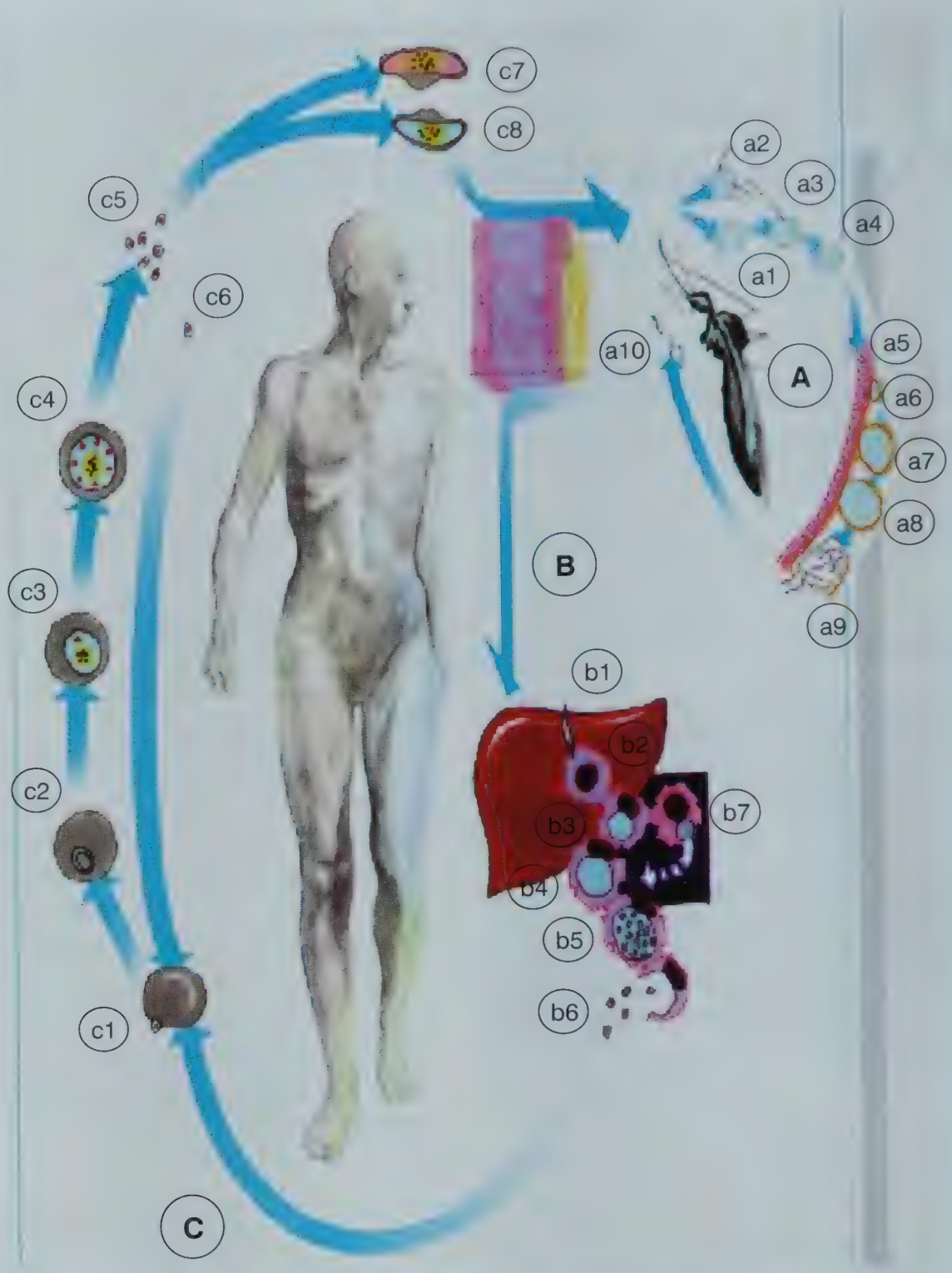

Fig. 1. Diagrammatic representation of the life cycle of Plasmodia affecting man.
A. Sporogonic cycle: Microgametes (a1) meet a macrogamete (a2) in the gut of the mosquito. Fertlizatio (a3) gives rise to the zygote (a4) which develops into an ookinete (a5). The ookinete penetrates the stom ach: on its outer wall the oocyst (a6) matures (a7 - a8) to release motile sporozoites (a9). Sporozoite reaches the salivary glands (a10) from which they can be injected into a vertebrate host.
B. Exoerythrocytic schizogonic cycle: Free circulating sporozoites (b1) penetrate the hepatocytes (b2). I the liver cells the schizogonic cycle takes place (b3 - b4) to produce schizonts (b5) and merozoites (b6 which are released into the circulation. In the case of P.vivax and P.ovale hypnozoites (b7) dormant para sites that may cause relapses later, are also formed.
C. Erythrocytic schizogonic cycle: Merozoites invade red blood cells (c1) and initiate the maturativ process developing into the ring-shaped early trophozoite (c2), the mature trophozoite (c3), and the sch izont (c4). Following the rupture of the erythrocytes the schizont releases the merozoites into the circula tion (c5). The merozoites re-invade new erythrocytes perpetuating the cycle (c6). Some trophozoites d not enter schizogonic division and develop into female (macro-) (c7) or male (micro-) (c8) gametocytes.
Reproduced, by kind permission of Masson Italia, from: Atlas of Human Protozoa, Rondonelli E. G Scoglia M., 1993.

There are four species of Plasmodium of medical interest: *P. vivax*; *P. ovale*; *P. malariae*; *P. falciparum*. Some characteristics of the four species of human Plasmodia are summarized in table 2. *P. falciparum* belongs to the subgenus *Laverania*, while the remainder three species of human malaria belong to the subgenus *Plasmodium.* Although subgeneric designation is rarely used, the distinction is important because it seems probable that the two subgenera present profound phylogenetic differences with relevant clinical significance.

The comprehensive life-cycle of the species of Plasmodium occurring in man is shown in diagrammatic form in figure 1. It comprises the exoerythrocitic (B1 to B7) and erythrocytic stages in the vertebrate host (C1 to C8) and the sporogonic cycle in the mosquito (A1 to A10). The humans and other vertebrates act as the intermediate host for the parasite, while the mosquito, in which the sexual reproduction takes place, is considered to be the definitive one.

The exoerythrocytic stages of malaria parasites in humans

Malaria infection in the human host starts when the **sporozoites** are injected into the blood stream during a blood meal by an infectious mosquito. Although it is assumed than one single sporozoite is capable of initiating the infection in men, the number of sporozoites injected by a mosquito bite is supposed to vary from dozens to thousands. It is likely that this number strongly affects the clinical picture: the greatest the sporozoite load, the shortest the incubation period and the most serious the symptoms. The sporozoites remain into the circulation for a short period, calculated as 60 minutes at maximum, before they actively enter the liver of the host (Lopez-Antunano, 1980)[1]. The Kuppfer cells in the liver may be invaded (or the parasite may be phagocitated) but the sporozoites are not able to develop in those cells and die shortly after invasion. Most parasites however invade the hepatocytes and start the asexual **exo-erythrocitic schizogonic cycle**. The cycle has been studied in details in liver sections from a rodent model for malaria infection, but observations from liver biopsis in human volunteers are also available for *P. falciparum* and *P. vivax.* The liver trophozoite initially appears as a mononucleated round body into the cytoplasm of the host cell; subsequently it begins to develop and multiply asexually, a mature **schizont** (the multinucleated stage of the parasite) is formed, and finally a large number of **merozoites** are released. The mature schizont is 30-70 μm large, has no pigment (there is no hemoglobin into the hepatocyte), and occupies the entire cell cytoplasm. The lenght of the schizogonic liver cycle is constant for each Plasmodium species to the extent that it can be considered a taxonomic character: 5.5 days, 8, 9, and 15 days for *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* respectively. The number of merozoites produced at the end of the cycle is also species dependant: it is estimated as 2,000 for *P. malariae*, 10,000 for *P. vivax/P. ovale*, and up to 30,000 for *P. falciparum* (Garnham,1966)[2]. The liver cycle ends when the mature schizont ruptures and releases the merozoites into the sinusoids of the liver. Released merozoites can only invade a red blood cell: the theory of the continuation of the liver cycle by invasion of a new hepatocyte by the merozoite, is not any more accepted at present.

Biological basis for relapses

Two species of human malaria determine a relapsing infection: *P. vivax* and *P. ovale*. In these two species some of the liver trophozoites immediately start the exo-erythrocytic schizogonic cycle which has been described above, while others remain into the liver in a latent (dormant) stage for varying periods of time and are termed **hypnozoites** (Krotoski, 1985)[3]. The lenght of the period of dormancy varies with the subpopulations of *P. vivax* and *P. ovale*: the relapse, in other words, is not triggered by host factors (waning of immunity) but rather seems to be a genetically determined intrinsic property of the parasite. A single inoculation of sporozoites of a relapsing species contains a mixture of genetically distinct parasites

that give rise to discrete subpopulations of exoerythrocitic trophozoites. The number of relapses, as their periodicity, seems to be a characteristic of the parasite strain. At one side of the spectrum of possibilities is the *P. vivax hibernans* strain who has a homogeneous population of sporozoites characterized by a latency of 250 days or more. At the other side is *P. vivax chesson* strain with an heterogeneus population of sporozoites, some programmed for immediate development, others to determine relapses at intervals of 2 to 3 months for a period of up to 2 years (Coatney, 1950)[4]. Actually, the two group of strains are considered to differ to the extent to justify their separation into two subspecies (Garnham, 1966)[2].

The erythrocitic stages of malaria parasites in humans

The erythrocitic stages of malaria parasites has several important implications in clinical practice: first, these are the only stages causing the complex and varying spectrum of symptoms characterizing the disease in humans; secondarily, the recognition of parasites in the blood of a patient allows the diagnosis of the infection and the differentiation of the various species of the causing agent.

The time required to complete the erythrocytic cycle is a fixed characteristic of the parasite species (see table 2): *P. falciparum*, and *P. vivax*, have a 48-hour development period, in *P. ovale* it lasts 50 hours, while *P. malariae* has a longer cycle of 72 hours. Theoretically the periodicity of the erythrocytic cycle would determine the classical cyclical presentation of symptoms every other day in tertian malaria infections and every third day in quartan malaria. In practice, however, the typical periodicity of malaria paroxysm cannot be recognized in the initial periods, since most parasite populations are heterogeneus and continuous fever therefore results from the completion of asynchronized schizogonic cycles.

The blood phase of the life-cycle is initiated when the merozoites from liver schizonts are discharged into the circulation (Garnham, 1988)[5]. The **merozoite** is 1 μm in diameter, consisting of a single nucleus and adjacent cytoplasm. It invades almost immediately an erythrocyte to enter its **trophozoite** stage. A vacuole is produce by the parasite which assumes the characteristic **ring form** (the young trophozoite). Within 12-24 hours, as the parasite grows, the cytoplasm expands, the vacuole slowly disappears and a characteristic parasitic pigment becomes visible within the cytoplasm. At the end of this phase the trophozoite has a single nucleus, a large cytoplasm, no vacuole, and a variable amount of pigment. The nucleus starts to divide approximately 30 hours after invasion in the case of *P. falciparum*, *P. vivax*, and *P. ovale*, while in *P. malariae* this requires approximately 40 hours. As nuclear division produces two or more nuclei the parasite enters the stage of a **schizont**. Nuclear division continues untill an appropriate number of merozoites is produced: approximately 36 for *P. falciparum*, 24 for *P. vivax* and *P.ovale*, 12 for *P. malariae*. At the end of this phase the schizogonic cycle is completed, the erythrocyte ruptures releasing the merozoites into the blood stream and determining the typical malaria paroxism. The merozoites discharged into the circulation invade new erythrocytes to repeat the schizogonic cycle untill the process is inhibited by the specific immune response or by chemotherapy. In the course of a schizogonic cycle (whithin a red blood cell) some of the merozoites become differentiated into sexual forms (the gametocytes); the mechanisms at the basis of this differential development are unknown. Gametocytes appears early (approximately from the third generation) in infections caused by *P. vivax*, *P. ovale*, and *P. malariae*, while at least 10 generation are thought to be required before *P. falciparum* gametocytes appears in the blood, which probably reflects the slow maturation and the sequestration of the immature stages in this species (Carter, 1980)[6].

The morphologic characteristics of the stages of malarial parasites in human blood are described in details in another section of the book. The first stage of the maturation process is the ring form. In *P. falciparum* (*) the ring form has variable size, the smallest are only 1.2 μm in diam-

eter, tend to adhere to the internal surface of the erythrocyte (accolè form, marginal form) and the nucleus is often divided to show two chromatin dots. As the cytoplasm becomes fleshy and the vacuole reduces in size, the parasites disappear from the peripheral circulation. The ring form is the only asexual stage usually identifiable in the peripheral blood of patients with *P. falciparum* infection (gametocytes may be present after the second week of patent asexual parasitaemia) because more mature stages of this species adhere to the endothelium of post-capillary venules in the tissues. *P. vivax* and *P. ovale* ring forms (*) have similar features, but the cytoplasm may early present amoeboid tendency and traces of stippling start to appear on the parasitized erythrocyte. In these species trophozoites are seen in the peripheral circulation: while the parasite mature the cytoplasm enlarges and presents amoeboid pseudopodes (the erythrocyte enlarges as well to contain the parasite), the vacuole disappears, the pigment (product of haemoglobin cathabolism) becomes prominent, and the stippling are profuse. *P. malariae* throphozoites (*) are regular in shape (except those assuming a characteristic band form accross the erythrocyte), with a small vacuole, early and abundant pigment, no stippling.

Mature schizonts in *P. vivax/ovale* present a large number of merozoites irregularly arranged and occupying the whole of the red blood cell, with more and more prominent stippling. In *P. malariae* a smaller number of merozoites are often regularly arranged around the central mass of pigment (daisy shape) and no stippling is present.

Gametocytes of *P. vivax/ovale* and *P. malariae* are morphologically similar to the late trophozoite: however, they present a completely matured parasite (no vacuole is visible) with abundant and unagglomerated pigment. *P. falciparum* gametocytes present a typical banana-like shape. Two types of gametocytes are found in the peripheral blood: the female macro-gametocytes and the male micro-gametocytes. They can be differentiated by the fact that in the male parasite nuclear material is dispersed (preparing to exflagellation) while in the female parasite it is condensed.

(*) See figures in the chapter on "Diagnosis of Malaria Infection".

The sporogonic cycle in the mosquito

Female mosquitoes seek a blood meal as a protein source for egg production. The cycle of blood meal and oviposition continues throughout the life of the female mosquito and requires repeated contacts with the vertebrate host, allowing for ingestion of malaria parasites, their multiplication and maturation, and transmission to other individual hosts during subsequent feedings.

The sporogonic cycle initiates when mature female and male gametocytes are ingested by a suitable species of Anopheles during a blood meal. As soon as gametocytes reaches the midgut of the insect the female gametocyte shed the red blood cell and remains free in the extracellular space as a **macrogamete**. The male gametocyte nucleus divide into eight sperm-like flagellated **microgametes** each of which also leaves the erythrocyte, reaches the midgut and actively moves to fertilize a macrogamete. Exflagellation of the microgametocyte is triggered by factors present in the mosquito midgut and begins about ten minutes after the blood meal. The result of the fertilization process is the **zigote**, which develops into the elongated, slowly motile **ookinete** whithin 18 hours from the blood meal. The ookinete actively penetrates the peritrophic membrane and the epitelium of the midgut and settles beneath the basal lamina of the outer gut wall, where it develops into a non motile **oocyst** 24-72 hours after the blood meal. The initial oocyst is 6-8 μm in diameter, has a single nucleus and a thin cist wall; the oocyst nucleus divides repeatedly leading to the formation of as many as 10,000 new individual nuclei whithin a mature oocyst of 40-60 μm in diameter. The time required from the establishment of the initial oocyst to its maturation depends on the parasite species, the

anopheline species and on the temperature: it varies from 7 to 30 days. The product of the mature oocyst are the **sporozoites**, narrow and curved in shape, actively motile, 10-15 μm in lenght. The sporozoites leave actively the cyst passing through small perforations without destroying the wall, at least till most of the parasites have been released, and move into the haemocelomic space of the insect. The sporozoite migrates and reaches the salivary glands (though most tissues may be invaded) where it penetrates the basal membrane, pass intracellularly through a secretory cell and settles into the salivary duct. Oocyst sporozoite are poorly motile and less infectious and immunogenic than salivary gland sporozoites, showing that significant changes occurr during the transfer journey into the hemolimph. When the mosquito feeds, the salivary fluid (which has anti-clotting properties) and its content of sporozoites are actively injected into the vertebrate host to start another asexual replicative cycle.

Temperature has an important effect on the speed of the cycle in the mosquito, who is a poikilothermic host. Development slows down and ceases for temperature of about 16°C with *P. vivax*; at very high temperature (about 45°C) the cycle is interrupted because the parasite dies. In general, the lenght of the sporogonic cycle in the mosquito is an irregular variable which strongly depends on the temperature and other climatic factors.

Terminology related to the life cycle of malaria parasites

At the light of the recent advances in the understanding of the life-cycle of malaria parasites, the terminology presented below has been widely accepted (Bruce-Chwatt, 1981)[7].

Prepatent period is defined the time from infection by mosquito bite until the time trophozoites are detectable in erythrocytes. The prepatent period is fixed and constant for each species of parasites. It has been calculated as 9-10 days in *P. falciparum*, 11-13 days in *P. vivax*, 10-14 days in *P. ovale*, and as long as 15-16 days in *P. malariae*. The prepatent period is associated with the lenght of the pre-erythrocytic cycle, which was measured in the past on volunteers inoculated with sporozoites by mosquito bite and detection of the circulating parasites by means of subinoculation of 500 ml of the volunteer's blood at regular intervals into a second group of volunteers, to test the blood infectiousness (Fairley, 1947)[8]. The lenght of the pre-erythrocytic cycle in humans have also been confirmed, in the case of *P. falciparum* and *P. vivax*, by studies on experimentally infected human volunteers who had a piece of liver removed by laparotomy at intervals after inoculation (Shortt, 1948; Shortt, 1951)[9, 10].

The prepatent period is longer than the pre-erythrocytic cycle, as several intraerythrocytic schizogonic cycles are necessary to build up detectable levels of parasitaemia.

Incubation period is defined the time from infection by mosquito bite until the first appearance of clinical symptoms. It is variable but usually much longer than the prepatent period as it requires parasitaemia to reach a sufficient density before symptoms occurr. The incubation period is thought to be influenced by the initial sporozoite load, and subsequently by the load of merozoites invading the blood stream: the higher the load the shorter the incubation period. A shorter incubation period is likely to be associated to a more severe disease, as symptoms arise when specific immunity, which starts developing when merozoites are discharged in the blood, is not yet built up.

Recrudescence is the renewed clinical manifestation of the infection due to persistance of erythrocytic forms into the circulation. This is tipically observed in *P. falciparum* infection exposed to subcurative drug treatment (latency of few days to few weeks) and in *P. malariae* (latency as long as several years).

Relapse is a renewed clinical manifestation of the infection started by persistent liver merozoites (hypnozoites) which start an exo-erythrocytic cycle months after the invasion of the hepatocyte. This is typically observed in *P.vivax* and *P.ovale* infection treated with drugs which have no action on the parasite liver stages.

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THE *ANOPHELES* VECTOR

F. Esposito, A. Habluetzel

Introductory remarks

Several insects are known to be vectors of human diseases. Mosquitoes in particular enjoy the questionable honour of having been the first insects to be associated with the transmission of a disease. This happened in 1878, when Manson, a British doctor practising in China, showed that mosquitoes (later identified as belonging to the genus *Culex)* transmitted human filariae. Likely, this finding influenced Ronald Ross who, in 1897, discovered oocysts on the gut wall of a mosquito fed on a malaria patient.

In 1898, the Italian zoologist G. B. Grassi and his coworkers first described the complete cycle of the human malaria parasites and pointed to a species of the genus *Anopheles* as responsible of malaria transmission. Since then we know that the epidemiology of malaria in a given environment is the result of a complex interplay among man, plasmodia and anopheline mosquitoes. These 3 elements have to be present for malaria transmission to occur in nature. *Anopheles* can be found – and actually they are – in areas where malaria parasites do not exist ("anophelism without malaria"), but malaria parasites need the vectors for perpetuating their life cycle. Artificially, human plasmodia can be transferred from infected to healthy individuals by blood transfusion or they can be kept indefinitely in culture in the laboratory, but their natural life cycle cannot dispense with the mosquito. The plasmodial infection seems to be neither advantageous nor detrimental for the mosquito, but is certainly detrimental for its human victims. The human organism is the natural shelter where malaria parasites thrive, multiply and differentiate in sexual forms. It represents at the same time the favorite source of bloodmeal for the females of several *Anopheles* species and this allows the relationship among the three "forced" partners contributing to the malaria cycle to be established.

Morphology and development

Various mosquito genera transmit plasmodia to different vertebrates, but all the vectors of human plasmodia belong to the genus *Anopheles*. A summary of mosquito taxonomy is presented in Table 1. Although taxonomy is a field for qualified specialists, the distinction between the most common mosquito genera – *Anopheles*, *Culex* and *Aedes* – is not too demanding and is of great practical importance.

The *Anopheles* egg is difficult to be seen by naked eye, being quite small (about 0.5 x 0.2 mm). It is boat-shaped and provided with two lateral floaters, filled with air (figure 1). The larvae hatch from the floating egg in form of small "shrimps", provided with a head, a thorax and nine abdominal segments (figure 2). The buccal apparatus is surrounded by brushes which create a wa-

Order	Family	Subfamilies	Genera
Diptera	Culicidae	Anophelinae 3 genera	*Anopheles, Chagasia, Bironella*
		Culicinae 33 genera	*Aedes, Culex, Mansonia, Culiseta, Haemagosus, Wyeomya*, etc.
		Toxorhynchitinae 1 genus	*Toxorhynchites*

Table 1. Classification scheme of Culicidae.

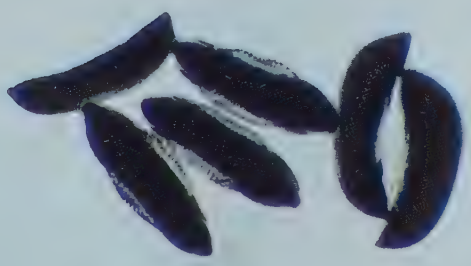

Fig. 1. *Anopheles* eggs showing the characteristic lateral floaters.

Fig. 2. Fourth instar larvae of *Anopheles*.

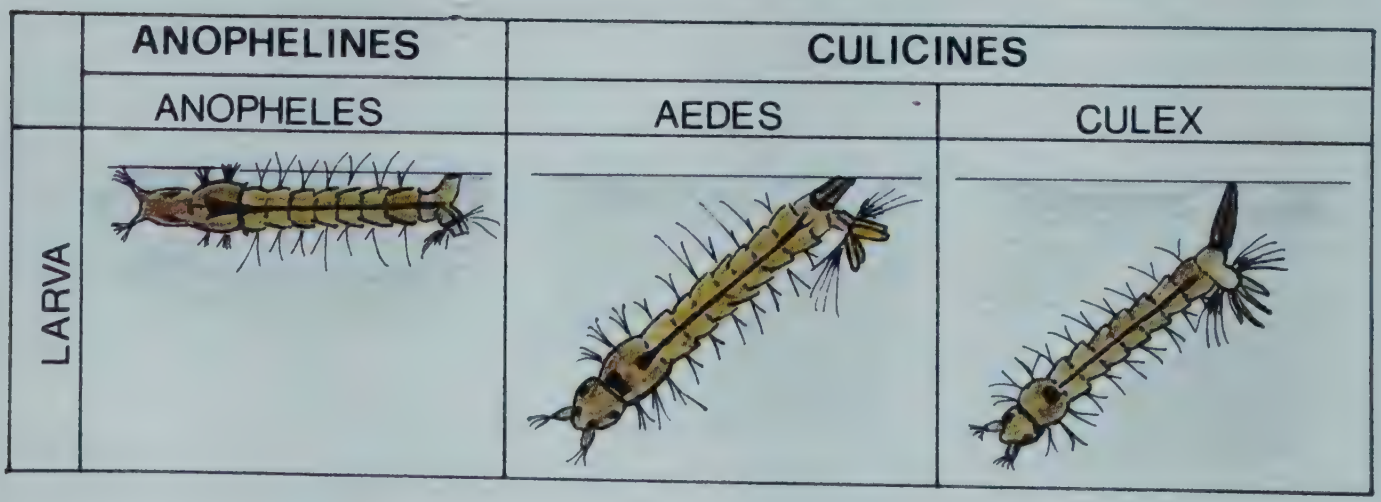

Fig. 3. The distinctive position of Anophelinae and Culicinae larvae with respect to the water surface (re-drawn from Bruce-Chwatt, 1985).

ter flow bringing food particles to the mouth. A conspicuous distinctive character is the position of the *Anopheles* larval body with respect to the water surface: parallel instead of obliquous like in *Aedes* and *Culex* (figure 3). This position might be related to the absence, in the *Anopheles* larva, of the respiratory siphon which in Culicine larvae brings the air to the tracheae.

The larvae undergo 3 successive moults, giving rise to organisms of a similar morphology, but of increasing sizes (from less than 1 mm for the 1st stage larvae to more than 0.5 cm for the 4th stage larvae). The fourth instar larva moults into a pupa, in which head and thorax are fused under a common envelope and bear the respiratory trumpets. The pupa is a relatively short stage during which the organism does not intake food. From it emerges the adult or

Fig. 4. Eclosion of an *Anopheles* adult from the pupa envelope (courtesy of Swiss Tropical Institute).

Fig. 5. Sexual dimorphism in *Anopheles* adults: a female on the left and a male on the right.

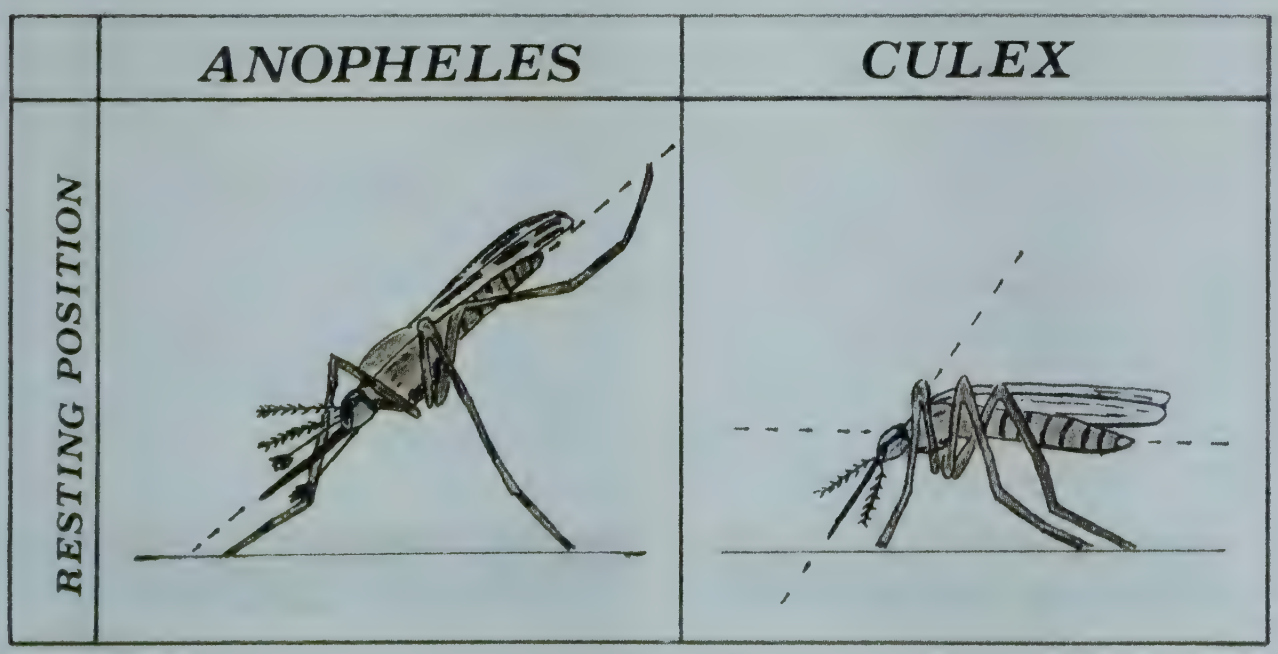

Fig. 6. The distinctive position of Anophelinae and Culicinae adults with respect to the substrate.

imago (figure 4). The duration of the whole process, from oviposition to adult eclosion, is temperature-dependent. At the elevated temperatures of the intertropical areas it takes a minimum of 7 days.

An apparent sexual dimorphism, particularly conspicuous at the level of the antennae, allows male and female *Anopheles* adults to be easily recognized (figure 5). The resting position, with the 3 main body parts (head, thorax and abdomen) characteristically aligned long a straight line allows the anopheline adults to be quickly distinguished from the culicines (figure 6).

Reproductive biology

Like in all living organisms, the reproductive biology occupies a crucial place in the life cycle of *Anopheles* mosquitoes. A nuptial flight is one of the first activities of the newly emerged mosquitoes. Males form swarms of variable size, which aggregate around a "marker" which make a contrast with the background (Muir, 1988)[1], e.g. the head of a person. Copulation frequently occurs within these swarms, but the significance of swarming in relation to copulation is still debated. After copulation, the female stores the sperms in a spermatheca, so that at each subsequent oviposition the eggs can be fertilized during their transit through the oviduct.

Male mosquitoes feed only on sugar-rich fluids, females may as well survive on these nutrients, but egg maturation requires the assumption of a bloodmeal by the female. Thus, "she" flies at dusk or, more usually, at night, looking for food for her progeny. Once an appropriate source is identified, the most suitable body regions of the prey are explored and a skin area where capillary blood vessels are particularly abundant and superficial is carefully and rapidly probed by the hydraulic engineering masterpiece represented by the mosquito mouth parts. When the "blood-fields" are reached, the pharyngeal pump is put into action and the formation of unwanted plugs of blood clots is avoided by anticoagulant substances contained in the saliva. The slender abdomen, characteristic of the newly emerged "empty" females, inflates like a baloon to accommodate a volume of blood which may even double the weight of the mosquito itself (1 to 3 µl, depending on the species, figure 7).

In the "freshly fed" females the abdomen is almost entirely filled with blood, but quite rapidly in the ensuing hours blood diges-

Fig. 7. A female taking the bloodmeal.

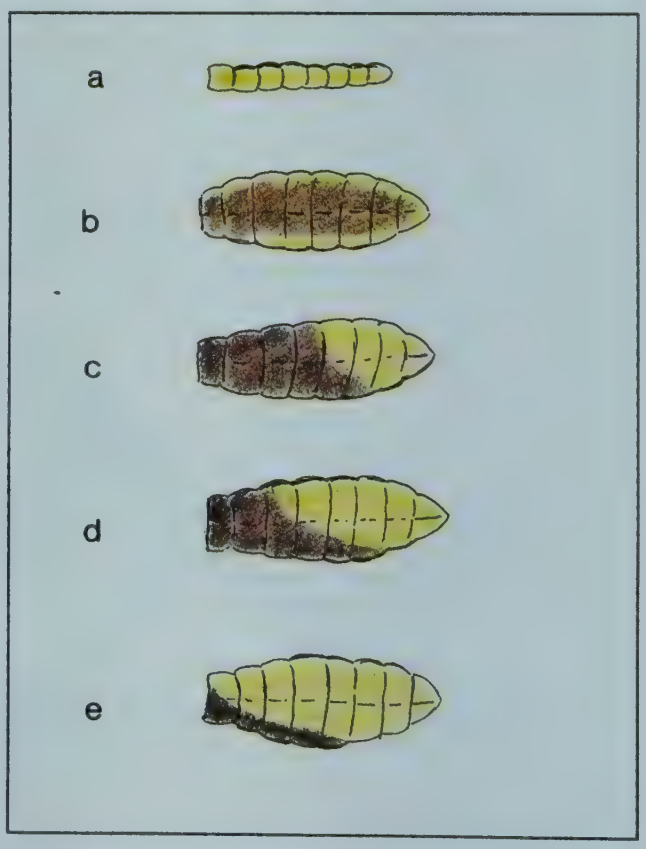

Fig. 8. The morphology of the abdomen in relation with the gonotrophic cycle development: lateral view of the abdomen of a) empty , b) freshly fed, c) and d) subgravid, e) gravid female.

tion entails the maturation of the eggs and a substantial increase of the volume occupied by the ovaries. The process is well discernible from the outside and, depending on the relative position of blood (or blood relics) and developing eggs, mosquitoes are defined as "subgravid" or "gravid" (figure 8). Usually, to lay the first batch of eggs a female mosquito requires 2 or even 3 bloodmeals, but subsequently blood taking and oviposition alternate regularly. The duration of the gonotrophic cycle, defined as the period between the bloodmeal and the subsequent oviposition, is temperature-dependent. When the average day-night temperature is above 23°C, the gonotrophic cycle takes 48 hours, which means that *Anopheles* females seek for a suitable source of blood every second to third night (Bruce-Chwatt, 1985)[2]. In areas where the proportion of *Plasmodium* gametocyte carriers is high, this implies that mosquitoes have a high likelihood of becoming infected sooner or later and, provided that the average mosquito life span is long enough for the sporogonic cycle of the parasites to be completed, this entails in turn high rates of human infection.

In this picture other factors play an important role, namely the preferred sources of blood and the location of the most frequent biting and resting sites. These characteristics are measured by parameters called anthropo(zoo-)phily, endo(exo-)phagy and endo(exo-)phily, respectively, and they are of primary importance for malaria epidemiology and for orientating malaria control strategies (see also the paragraph Methods in malariological entomology). Different *Anopheles* species also vary greatly in their preference for particular breeding sites. The main features of some important malaria vectors are given in the next paragraph.

Distribution and characteristics of some important *Anopheles* vectors

In the following pages the main characteristics of some *Anopheles* species and species complexes are summarized, more as an example than as an exhaustive guide which would be beyond the scope of this handbook and far beyond the competence of the authors of this chapter. The species problem in *Anopheles* is a fascinating zoological and genetic puzzle and has been reviewed by Coluzzi (1988)[3]. Here we give a brief outlook on morphology, geographical distribution, habitat and behaviour of 10 species or species complexes considered amongst the most important malaria vectors all over the world.

a) *Anopheles gambiae* complex

It includes 6 sibling species which can be identified on a cytogenetic basis or using the methods of molecular genetics. A specimen of *A. gambiae* s.s. ([1]) is shown in figure 9. This mosquito is extremely well adapted to human settings, as witnessed by its remarkable anthropophily and en-

([1]) s.s. = *sensu stricto*, this is one of the 6 sibling species of the complex and is opposed to s.l. = *sensu lato*, meaning anyone of the 6 sibling species member of the *An. gambiae* complex.

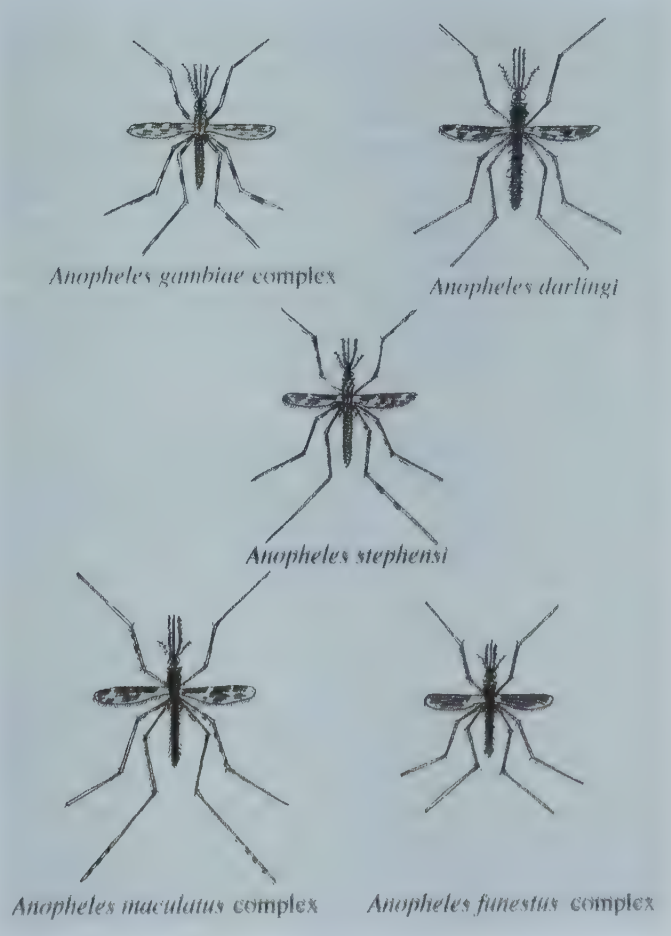

Fig. 9. Some important malaria vector species.

dophily, which make it one of the most efficient malaria vectors. This species is found all over tropical Africa and is sympatric with the less strictly anthropophilic *A. arabiensis*, which is found in South-West Arabia as well. Both species like breeding sites represented by sunny and clean water pools, almost devoid of vegetation. Particularly important are man made breeding sites, like wells digged to prepare mud bricks. Typically, small water pools created by cattle footprints or grooves tracked by vehicle tyres are suitable breeding sites for these malaria vectors. *A. melas* and *A. merus*, less important vectors, are adapted to brackish waters in West and East Africa, respectively.

b) *Anopheles funestus* complex

This complex of small, dark species represents the second important African vector complex (figure 9). Sympatric with *A. gambiae* s.s. and *A. arabiensis*, these mosquitoes succeed to the members of the *A. gambiae* complex in the colonization of the breeding sites. For reasons which are not totally clear (competitive exclusion?), towards the end of the rainy season, when the densities of the *A. gambiae* complex mosquitoes start decreasing, the grassy edges of the water pools become the favorite breeding sites for *A. funestus* mosquitoes. Anthropophilic and endophilic, they are powerful vectors which may promote the extension of the malaria transmission season to the first months of the dry season. Due to the reduced size and the dark colour, they can be easily distinguished from vectors of the *A. gambiae* complex.

The *A. minimus* complex appears to be closely related to the *A. funestus* complex. It includes a few important vector species in India and South-East Asia.

c) *Anopheles maculipennis* complex

These mosquitoes are closely related to the species which were responsible for malaria transmission in Europe (*A. sacharovi*, *A. atroparvus* and *A. labranchiae*), before the eradication of the disease. Although still present in some European countries (Greece, Italy), nowadays they may act as vectors of malaria in limited areas of Iraq and Turkey (particularly in Kurd refugee camps). Their degree of anthropophily varies, but they tend to take their bloodmeals outdoors and to rest indoors. The breeding sites are represented by sunny pools rich in vegetation, including brackish marshes.

d) *Anopheles stephensi*

An important urban and rural vector (figure 9), widely distributed from Afghanistan to China, India, Pakistan, Iraq, Iran, Saudi Arabia. It finds appropriate breeding sites both in urban peridomestic water collections and in rural pools. In town it is mainly anthropophilic and endophilic, while the rural sub-populations are more variable in their habits.

e) *Anopheles culicifacies* complex

As its name says (*culicifacies* = *Culex*-like), the complex includes at least 4 sibling species which in some aspects may recall culicines mosquitoes. Partly sympatric with *A. stephensi*, it extends its distribution to South-East Asia (Laos, Thailand, Vietnam) and to East Africa (Ethiopia). Its biting preference for cattle renders it sometimes an inefficient vector, which may become important when reaching very high densities. Quite flexible in biting and resting habits, it may bite indoors and outdoors and rest in human and animal shelters.

f) *Anopheles maculatus* complex

Likely, the species composing this complex have different ecological distribution and behaviour and this may explain why these mosquitoes are a vector in some areas and not in others (figure 9). Its distribution extends from China and India to South-East Asia. Its breeding sites are sunny streams, often produced by deforestation, and feeds mainly on cattle. It may be forced to bite on humans when other sources of bloodmeal are not available.

g) *Anopheles punctulatus* complex

Species of this complex are found in different habitats of Papua New Guinea, the Solomon Islands and Vanuatu. Coastal ar-

eas are colonized by *A. farauti*, the central plains of New Guinea by *A. koliensis*, and the shady forest hills by *A. punctulatus*. Mosquitoes of this complex feed on humans, but are easily diverted on cattle. The resting habits are quite flexible: a trend to endophily can easily be reverted to exophily, for instance when houses are sprayed with DDT. The numerous spots on the wings (*punctus* = spot) are characteristic of these mosquitoes.

h) *Anopheles albimanus*

This vector of malaria in Central America, the Antilles and Caribbean can be found also in Texas and Florida. It may adopt a wide spectrum of breeding sites, from artificial reservoirs to pits, marshes and lagoons. This mosquito displays a very flexible attitude with respect to feeding and resting habits. Small and dark, it is characterized by pale hind legs (*albimanus* = white handed).

i) *Anopheles darlingi*

Found in practice all over Central and Southern America (figure 9), it likes various kinds of water collections as breeding sites, provided that they are protected with vegetation. The most endophilic fraction of the population having been eliminated by antivectorial operations, it behaves now like an "opportunistic" vector, occasionally biting humans mainly outdoors, in forest areas. Of course these characteristics have diminished the importance of *A. darlingi* as a vector of malaria, but the deforestation activities in the Amazons are causing its replacement by the much more efficient members of the *A. gambiae* complex.

Methods in malariological entomology

Because of the role of the *Anopheles* vector in the malaria cycle, a trustable analysis of the malaria situation in a given area and a sound programme of malaria control cannot dispense with entomology. Without entering the complexity of the mathematical relationships describing the rules which allow the intensity of malaria transmission to be "predicted" on the basis of measurements of several parameters, we try here to summarize the methods which allow the most important data describing the entomological component of the malaria cycle to be collected.

Fig. 10. Three models of mosquito-collecting aspirators: the simplest mouth-operated model, the paper cup aspirator of Coluzzi & Petrarca (1973)[4], and its modified electric-powered version.

An apparently simple but fundamental parameter to be measured is the *Anopheles* density, which means the average number of mosquito vectors per inhabitant. The reference method for measuring vector density is the so-called landing or human bait catch. With the help of a simple aspirator (figure 10), mosquitoes are caught when they land on a human volunteer for taking a bloodmeal. The rationale for the choice of this method is straightforward and is based on a few considerations. The feeding and resting habits of *Anopheles* not only vary both inter- and intra-specifically, but they may also vary under the selective pressure exerted by intervention measures. Coluzzi and coworkers (1979)[5] have shown that certain karyotypes of *A. arabiensis* are found with different frequencies biting outdoors or indoors and *A. farauti* shifted from prevailing endophily to exophily after indoor spray operations were implemented in Solomon Islands (Taylor, 1975)[6]. It would be therefore risky to determine the *Anopheles* density using the results of catches performed at the presumed resting sites. In addition, the fraction of the mosquito population which actually "counts" in terms of malaria transmission is constituted by the females who come for biting, thus it seems very

Fig. 11. Scheme illustrating the mechanism of action of the CDC light trap.

appropriate to target the catch method to these mosquitoes. Practical and ethical considerations however argue against the human bait catches. From the practical point of view, since most malaria vectors bite at night (sometimes late at night), landing catches are very labour demanding in terms of execution and supervision. From the ethical point of view, the deliberate exposure of human volunteers to mosquito-borne diseases (not only malaria) is definitely questionable. Alternatives have been proposed, like the human baited double bednet: a first bednet is partly uprisen or on purpose torn and works as a trap, while the second is properly located and protects the volunteer from being bitten.

In recent years, a light trap designed at the Center for Diseases Control in Atlanta, USA, has become increasingly popular (figure 11). Located near a volunteer sleeping under a bednet, at a height of about 0.5 m from the ground, the trap exploits the attractiveness of the human bait "from far". When the mosquito approaches to bite, the light of a small battery operated bulb may exert an attraction "from near" and bring the insect close to the fan which aspirate it into the trap bag. Practical and independent on the collector skill, the CDC light traps are definitely less efficient than the human bait catch. This may lead to an underestimation of the real amount of transmission in baseline epidemiological studies, but does not represent a major disadvantage in control programmes where usually either historical or parallel reference data for measuring the impact are available.

For special purposes, the human bait (in landing or in CDC trap catches) can be substituted by animal baits, for instance when sampling the least anthropophilic or the zoophilic fraction of the population is required.

When the *Anopheles* populations thriving in a certain area are quite stable in their habits, and their resting places are well known and easily accessible, other methods can be used. The same kind of aspirators previously shown can be used to collect living mosquitoes at their resting places (figure 12). For strictly endophilic vectors another practical and widespread method consists in spraying a pyrethrum suspension in a closed room/house. The knocked-down mosquitoes can then be collected on a white sheet previously laid on the floor (figure 13).

The sampled mosquito specimens, whatever catching method is used, can be sorted by sex, species (e.g. *A. gambiae* s.l. and *A. funestus*), stage of the gonotrophic cycle (empty, freshly fed, subgravid, and gravid) depending on the study aim, and stored for further analyses.

In case the mosquito sample is not obviously fed on humans, it may be interesting to determine the origin of the bloodmeal to evaluate host preferences. This parameter is largely influenced by host availability, however clear-cut differences for host-specific tendencies have been demonstrated. When humans and cattle were equally made available to *A. merus* and *A. gambiae*, the former took the majority of meals on animals and the latter on humans (Gillies, 1967)[7], indicating that a primarily ecological adaptation had become genetically fixed. Bearing in mind that these habits can be partially or totally reverted by host availability, various *Anopheles* species can be ranked according to their degree of anthropophily, measured by the human blood index, i.e. the proportion of mosquitoes fed on humans (Table 2).

Various methods can be applied to examine the midgut content of freshly fed mosquitoes (Pant *et al.*, 1990)[9]. We briefly

Fig. 12. Catching mosquitoes at suspected resting places, the "canari" where drinking water is stored.

Fig. 13. Recovering mosquitoes on the sheet after a pyrethrum spray catch.

outline here (figure 14) a simple immunoenzymatic (ELISA) method described by Habluetzel and coworkers (1988)[10].

The abdomens of freshly fed mosquitoes, which can be stored dry for months at room temperature, are crushed in a proper buffer. The resulting homogenate is transferred to a 96-well microplate. Among the proteins sticking to the wells are the immunoglobulins (Ig) of the animal on which the bloodmeal was taken. By using animal-specific antisera directed against the Ig fraction, it is possible to identify the bloodmeal source. When only the determination of the anthropophilic fraction of the population (i.e. the human blood index) is of interest, which is most frequently the case, an antiserum anti-human Ig can be used. In these conditions, the ratio positive-over-tested represents directly the human blood index of the studied sample. The importance of this parameter resides in the fact that it is present at the square power in the formula quantifying the "power" of a vector (vectorial capacity) for transmitting malaria (Macdonald, 1957)[11]. This means that the vectorial capacity of a fully anthropophilic vector is 4-fold that of an *Anopheles* population which takes only one half of the bloodmeals on humans. This can be easily understood by observing that the alimentary preference enters twice in the determination of the efficiency of a vector: once when it takes a bloodmeal infecting itself, and a second time when it takes a bloodmeal infecting the human host. By applying the multiplicative rule of the probability, it appears that a mosquito having a 50% probability of biting on humans, has a probability of 25% of accomplishing two "successful", independent events (i.e. of biting twice on humans), compared to the 100% of a strictly anthropophilic vector.

A third important parameter in malariological entomology is the survival time of the mosquito. Under favorable environmental conditions, an *Anopheles* mosquito can live for more than 3-4 weeks, but the mean survival in nature is much shorter (6-9 days). Thus, only a relatively small fraction of the population lives long enough for the sporogonic cycle to be accomplished. The duration of the sporogonic cycle depends on the parasite species and on the environmental temperature, its minimum being of 10 days. This stresses the importance of age-grading a mosquito population, in order to estimate the proportion of females who live long enough to transmit malaria. Age-grading is crucial in malaria control programmes targeted at the adult vector (e.g. indoor spraying or impregnated bednet/curtains). The result of these control activities – if successful – is a shortening of the vector population mean sur-

HBI > 0.5	0.1 < HBI < 0.5	HBI < 0.1
A. farauti	*A. culicifacies*	*A. algeriensis*
A. funestus	*A. messeae*	*A. aconitus*
A. gambiae s.l.	*A. quadrimaculatus*	*A. annularis*
A. koliensis	*A. rufipes*	*A. mascarensis*
A. nili	*A. sergentii*	*A. multicolor*
	A. sinensis	*A. subpictus malayensis*
	A. sundaicus	

Table 2. Human blood index (HBI) of various *Anopheles* – modified from Garret-Jones (1964)[8].

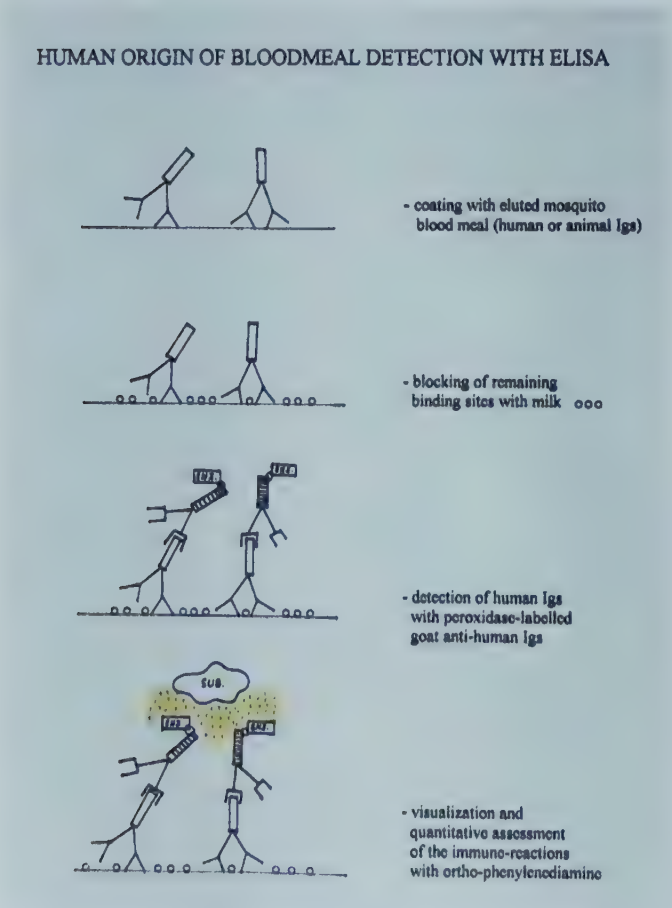
HUMAN ORIGIN OF BLOODMEAL DETECTION WITH ELISA
- coating with eluted mosquito blood meal (human or animal Igs)
- blocking of remaining binding sites with milk
- detection of human Igs with peroxidase-labelled goat anti-human Igs
SUB.
- visualization and quantitative assessment of the immuno-reactions with ortho-phenylenediamine

Fig. 14. Scheme of the ELISA test for identifying the origin of the mosquito bloodmeal.

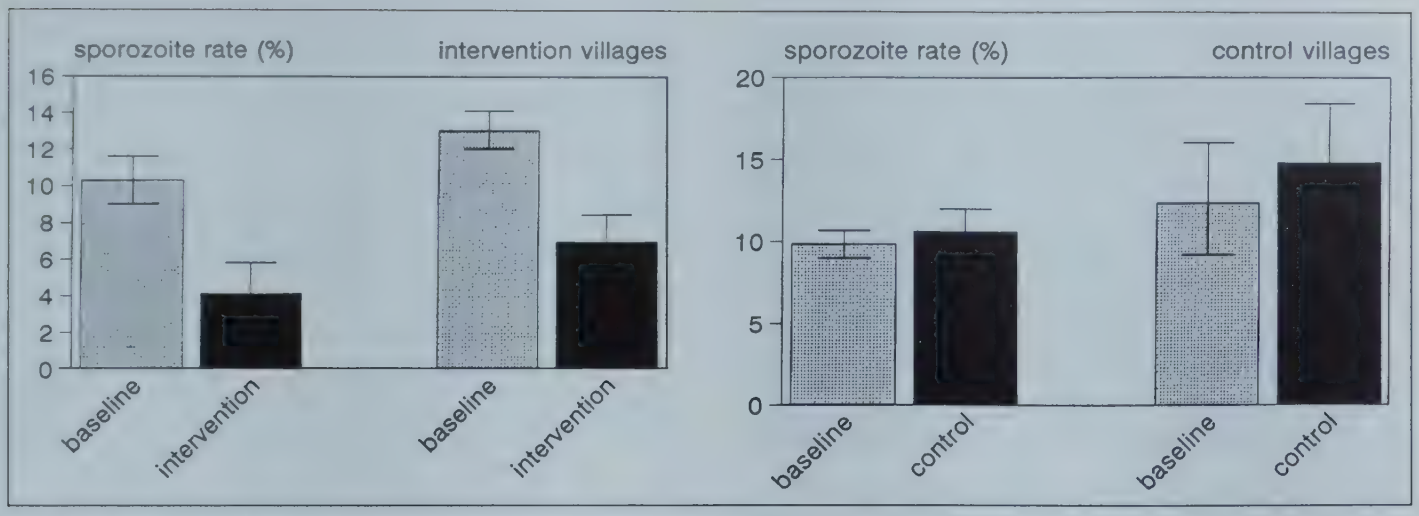

Fig. 15. Impact of insecticide impregnated curtains on the sporozoite rate: results obtained in 2 intervention villages and in 2 control villages are shown, respectively. The grey columns refer to the baseline year, before the intervention, and black columns to the intervention or control year (unpublished data of A. Habluetzel and coworkers).

vival or, in other words, an increase of its daily mortality. We have seen before that most anophelines, in the absence of limiting factors and in favorable climatic conditions, take a bloodmeal and accomplish a gonotrophic cycle every 2nd to 3rd day. If the relevant vector is an endophilic one and if a malaria control programme using e.g. insecticide-impregnated curtains is at work, each time the mosquito enters the house it has a certain chance of getting in contact with the insecticide and of being killed. As a consequence, the "life expectancy" of that mosquito may be dramatically shortened. If the control measure is effective, this shortening may have an impact on the sporozoite rate, i.e. on the proportion of mosquitoes harbouring *Plasmodium* sporozoites in their salivary glands. This has been the case in a malaria control programme which is currently run in Burkina Faso (figure 15).

The determination of the sporozoite rate was classically done by dissection of the salivary glands and microscopic examination of their content. This time consuming and skill demanding method, which could be applied only on freshly collected (preferrably living) samples, has been replaced by an immunological method developed by Zavala and coworkers (1982)[12]. The method, now widespread in its ELISA variant (figure 16), is based on monoclonal antibodies detecting a peculiar antigen of the sporozoite surface – the circumsporozoite (CS) protein – and allows dried specimens to be used years after their collection and the infecting *Plasmodium* species to be identified. It has been pointed out that the presence of the CS antigen does not nec-

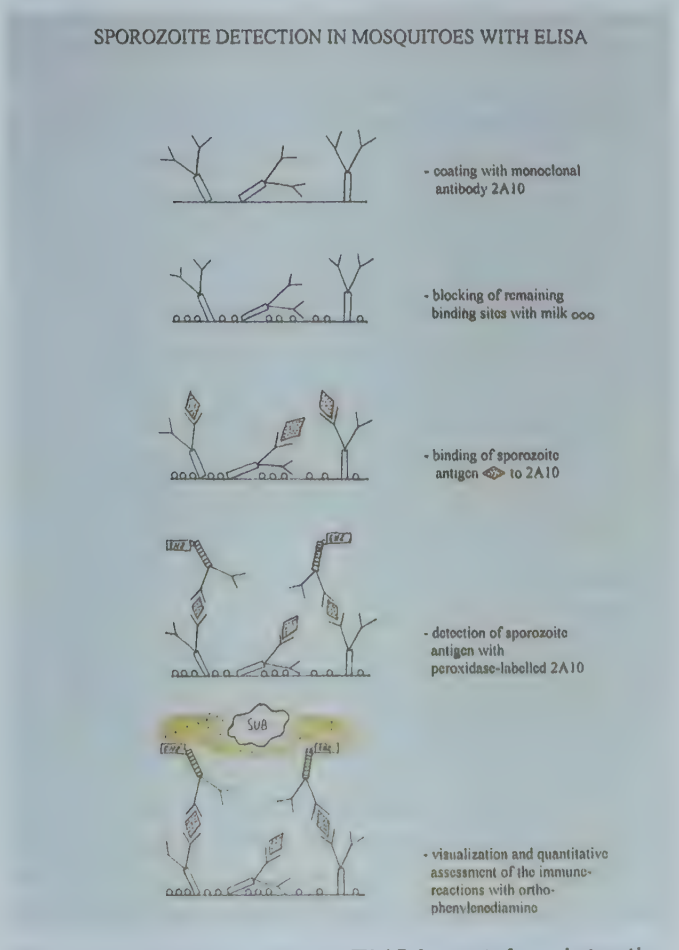
SPOROZOITE DETECTION IN MOSQUITOES WITH ELISA
- coating with monoclonal antibody 2A10
- blocking of remaining binding sites with milk ooo
- binding of sporozoite antigen to 2A10
- detection of sporozoite antigen with peroxidase-labelled 2A10
SUB
- visualization and quantitative assessment of the immune-reactions with ortho-phenylenediamine

Fig. 16. Scheme of the ELISA test for detecting the sporozoite antigen in malaria vectors.

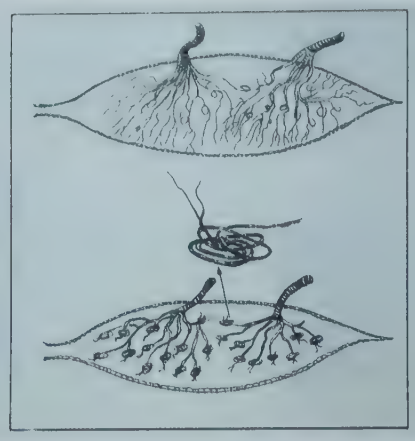

Fig. 17. The skeins of the ovariolar tracheoles (indicated by the arrow) are characteristic of nulliparous females, and allow them to be distinguished from parous specimens (lower part), where tracheoles appear stretched.

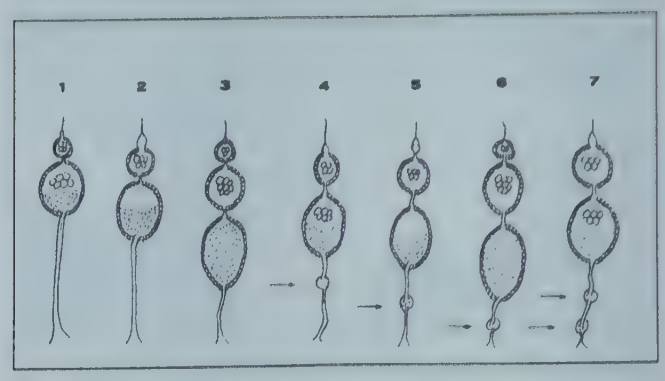

Fig. 18. The ovariolar dilatations (arrows) are thought to represent previous oviposition events.

essarily coincide with the presence of living sporozoites and that the ELISA results may therefore represent an overestimate of the true sporozoite rate (Lombardi *et al.*, 1987)[13]. The formal objections can be overcome by expressing the results as the proportion of infected mosquitoes (rather than infecting) and the discrepancy between ELISA and dissection can be minimized by processing in the assay the thorax of the mosquito rather than its whole body.

The sporozoite rate determination gives only an *a posteriori*, indirect and rough idea of the age structure of the vector population: indeed it tells us how many mosquitoes lived *at least* 10 days. Methods exist which directly assess morpho-physiological characteristics directly related to the mosquito age (Muir, 1988)[1]. The unravelling of the tracheoles of the ovaries associated with follicle growth allows the nulliparous females, who never laid eggs, to be distinguished from the parous ones (figure 17), who underwent already one or more gonotrophic cycles. The ratio parous/nulliparous is obviously related to the probability of vector survival, as parous specimens are certainly older than nulliparous, but requires repeated measures on large samples, being strongly influenced by short term meteorological variables. The almost simultaneous emergence of young adults from the breeding sites, as frequently occurs during the rainy season, may suddenly, i.e. from one day to the next, increase the nulliparous fraction. In addition, the partition between the 2 age categories – parous and nulliparous – occurs at an age (4-5 days) which is not really crucial in relation to malaria transmission. More detailed and useful information can be gathered from the dissection and microspical examination of the dilatations of the ovarioles (figure 18). These dilatations are thought to represent the "footprints" of previously laid eggs. Assuming a constant (and known) time interval between subsequent ovipositions, by multiplying this time by the number of dilatations the age of the mosquito could be estimated. Applying this method, Gillies (1988)[14] found for a Tanzanian population of *A. gambiae* that 65% of the mosquitoes died within the first week and 92% within a fortnight. Doubts on the interpretation of the dilatations have been raised by Hoc and Charlwood (1990)[15]. In addition, the method is very elaborate and to process a limited number of specimens substantial amount of time and remarkable skills are required. We estimate that for most practical purposes the determination of the sporozoite rate may be adequate.

Vector density, human blood index and sporozoite rate allow the entomological inoculation rate (EIR) to be calculated. The EIR is given by the density (number of vectors per inhabitant) multiplied by the human blood index and by the sporozoite rate. If we assume for instance that the

catches provided a number of 15 *Anopheles* per inhabitant and per night, that 78% of them were human fed and that 10% of the vectors showed to be sporozoite infected, the EIR will be 15 x 0.78 x 0.1=1.17. These are real figures from data collected by the authors in Burkina Faso (West Africa). This means that each individual receives 1.17 infective bites per night. The EIR is an useful parameter, since in a single figure it resumes the intensity of malaria transmission.

Conclusion and acknowledgements

In this chapter we tried to outline some practical aspects of entomology for those who, not being specialists, are interested in malaria epidemiology and control. We are fully aware that we could not provide all the details required to convert the notions we tried to illustrate in practical activities. Our laboratory is ready to provide further assistance to all the interested users of this handbook.

We are indebted to Mr Luciano Pasqualini and Mrs Roberta Maggiori for their excellent technical assistance and to Prof. Mario Coluzzi for critically reading the manuscript.

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EPIDEMIOLOGY OF HUMAN MALARIA PLASMODIA

A. E. C. Rietveld, R. L. Kouznetsov

Introduction

Malaria is a common and serious tropical disease. It is a public health problem in some 90 countries worldwide, inhabited by nearly 40% of the world population, i.e. over 2 billion people (1993 figures). It has been estimated that malaria incidence may be in the order of 300-500 million clinical cases globally each year. Countries in tropical Africa account for more than 90% of these. Estimates of malaria mortality vary from 1.5 to 2.7 million deaths worldwide per year, with some 100.000 deaths occurring outside Africa.

In this chapter, human and parasite factors influencing malaria transmission will be presented, followed by an example of how these factors intertwine in the historical evolution of the malaria distribution over the last century. In the second half of the chapter the present geographic distribution of the malaria problem is described along with the epidemiology of drug-resistant malaria. The chapter concludes with epidemiology in practice: major epidemiological types, high risk population groups, management information needs for malaria control, and the bare epidemiological necessities for district health officers.

Human malaria parasites and factors influencing malaria transmission

Plasmodium parasites, people, *Anopheles* mosquitos, and the environment all have a profound influence on the transmission potential for malaria infection. Some of the factors influencing transmission are inherent to the malaria species, their mode of transmission and the physical environment. Other factors are man-made, including development and malaria control efforts, which can have positive or negative effects on the transmission potential. Man-made factors such as war, migration, vector resistance to insecticides and parasite resistance to drugs invariably enhance malaria transmission, or at least make its control more difficult and expensive.

a) Malaria species and mode of transmission

Four species of *Plasmodium* protozoa cause malaria in humans: *P. falciparum, P. vivax, P. ovale* and *P. malariae*. The species differ in many ways, some of which have a direct bearing on transmission and distribution patterns.

Malaria parasites are normally transmitted from one person to another through infective bites of *Anopheles* mosquitos. The source of infection can be either a

sick person or an otherwise symptomless carrier of parasites. The form of the parasite that can infect mosquitos is the gametocyte. Gametocytes start developing in red blood cells in capillaries of the inner organs of infected persons after the invasion of the blood by merozoites. Mature gametocytes appear in the peripheral blood some 3 days (*P. vivax*) to 10 days (*P. falciparum* and *P. malariae*) later, ready to be picked up by a mosquito. In *P. vivax* and *P. ovale* infections a non-immune patient will thus be infectious to mosquitos at or shortly after the onset of clinical symptoms. In *P. falciparum* infections the patient becomes infectious only after several days when the mature gametocytes appear in the peripheral bloodstream. Persons in highly endemic areas who have been repeatedly infected with malaria acquire a degree of immunity to malaria which suppresses most clinical symptoms. These people may still carry gametocytes in their blood that will infect the mosquitos biting them. Antimalarial drugs such as chloroquine and mefloquine that are prescribed to cure malaria infections do not eliminate mature *P. falciparum* gametocytes from the bloodstream. A person who has been successfully treated with antimalarial drugs may thus be healthy but infective for up to two months until the *P. falciparum* gametocytes die off naturally, or until another drug such as primaquine is given that does eliminate the mature gametocytes.

The incubation period in malaria covers the time between infection and first appearance of clinical signs, of which fever is most common. The length of the incubation period depends on the infecting species and is usually between 9 and 30 days, shortest for *P. falciparum*, longer for *P. malariae*. In some strains of *P. vivax* the incubation period may last 8-9 months: perfectly suited to seasonal transmission in colder climates with a short transmission season.

In the case of *P. vivax* and *P. ovale*, some parasites remain dormant in the liver for many months. Relapses caused by these persistent liver forms (hypnozoites) may appear months, and occasionally up to 4 years after exposure, bringing with it the risk of delayed transmission of the infection to others. *P. malariae*, like *P. falciparum*, does not have persistent liver forms. However, untreated or partially treated blood infection with *P. malariae* may be present for many years, perhaps for life with or without clinical symptoms. Untreated *P. vivax* and *P. ovale* infections may linger on (and remain infectious) for 1 1/2 to 5 years, untreated *P. falciparum* 1-2 years. In areas with emerging drug-resistant *P. falciparum*, recrudescences of the infection may occur up to a month or more after what initially seemed to be a successful clinical cure.

Malaria can also be transmitted via blood transfusions. This can accidentally happen if a person donates blood before the onset of clinical symptoms, after merozoites have entered the blood stream from the liver. Blood donations from *P. malariae* carriers and (semi)immune persons without clinical symptoms may also contain malaria parasites. In certain cases this has given rise to malaria outbreaks in paediatric wards of urban hospitals, most notably concomitant with dengue haemorrhagic fever epidemics when many children receive transfusions. Similarly, malaria may be transmitted by contaminated needles and syringes. Relapses do not occur in relation to transfusion malaria. In congenital malaria, which is comparatively rare, parasites are transmitted from mother to child before and/or during birth.

All non-immune persons, regardless of age or sex, are susceptible to malaria infection. The only exceptions are the indigenous populations of Western and to some extent Central Africa, whose erythrocytes lack the Duffy blood group antigens which are essential for penetration of *P. vivax* into erythrocytes. Other genetic anomalies such as the sickle cell trait, ovalocytosis, glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, and thalassaemias alter but do not restrict malaria transmission. They may offer some protection against severe falciparum malaria.

b) Ambient temperature

Temperature is an important factor which, through its effect on the development of the malaria parasite in the vector

and its effect on the development of the vector itself, greatly influences the geographical distribution of malaria transmission in general and malaria parasite species in particular. Female *Anopheles* mosquitos ingest malaria gametocytes when taking a blood meal from an infected person. The parasite needs a period of development in the mosquito before it can infect other people again. The length of this extrinsic sporogonic cycle depends on the *Plasmodium* species and the ambient temperature. The development of *P. falciparum* in the female adult *Anopheles* mosquito requires a minimum temperature of 20°C whereas the other human malaria species can develop at temperatures down to a minimum of 16°C. Above the minimum temperature the development of the parasite in the vector accelerates with increasing temperature. The duration of sporogony at optimum temperatures for *P. falciparum* is 8-10 days. It is somewhat less for *P. vivax*. Sporogony of *P. malariae* and *P. ovale* takes longer, and only a minority of vectors live long enough to transmit them. Malaria epidemics may occur when conditions for malaria transmission are marginal in terms of temperature, rainfall patterns or altitude.

c) Development

There are many instances where socio-economic development has reduced malaria transmission through improvements in agriculture, housing construction, land use, etc. The concomitant development of health services will reduce morbidity and mortality and contribute to a reduction in transmission. However, uncoordinated development such as in slum areas of rapid urban growth may enhance malaria transmission. In ill-designed development projects the thoughtless creation of additional mosquito breeding places may combine with large-scale migration to cause malaria epidemics.

d) Control efforts

Control efforts aiming to substantially reduce transmission through unsustainable methods may put populations at risk of epidemic malaria when control efforts can no longer be kept up. Such may be the case in areas where the level of endemic malaria has been reduced through time-limited large-scale vector control programs.

e) War

Some recent epidemics occurred in areas of social and political upheaval or economic distress, where health infrastructure was destroyed, control efforts abandoned, and large populations had to leave their homes in search of security.

f) Migration

Migration of non-immunes into endemic areas may lead to malaria epidemics, as may migration of parasite carriers into non-endemic, receptive area. Receptivity refers to an abundant presence of anopheline vectors and the existence of other ecological and climatic factors favouring malaria transmission. Thus resettlement schemes and development projects involving an influx of labour forces may lead to malaria epidemics, as may refugee movements.

g) Resistance to drugs

Ineffective antimalarial drugs will not clear the parasites from the blood, thus potentially prolonging the infective period of patients. Also, as second line drugs tend to be more expensive, many people may no longer be able to afford a full treatment course.

Transmission patterns – two extremes

a) Stable malaria

Is caused by the presence of a vector with a frequent man-biting habit, with moderate to high longevity, at temperatures favourable to rapid completion of the sporogonic cycle. With such characteristics, only very few bites per person per night are necessary to maintain transmission. Seasonal changes will occur with changes in temperature. Transmission stops when the ambient temperature drops below some 15°C. A seasonal in-

crease may occur during the warm weather, starting at a relatively low temperature and terminated by low temperature. Marked fluctuations other than normal seasonal changes are not likely except when due to obvious causes. Epidemics are very unlikely, except among non-immune populations arriving to the area. The regularity of transmission is likely to ensure stable immunity, varying in degree from place to place. Only the young children will not have experience with malaria and thus no resistance to it[1]. In areas with stable malaria it is very difficult to reduce transmission. The first objective will be to reduce morbidity and mortality through early diagnosis and adequate treatment of malaria disease, especially in children, and through personal protection methods.

b) Unstable malaria

Occurs where malaria is transmitted by a vector which feeds on humans only infrequently. Consequently, high densities of *Anopheles* are needed to maintain transmission. Endemicity and the immunity status of the population will differ markedly by area and over time, depending on the presence of other factors favourable for propagation of mosquitos and thus malaria transmission. When conditions for transmission become favourable, epidemics may occur.

Epidemic malaria is the "malaria of non-immunes"[2]. Epidemics occur when non-immune and partially-immune populations are exposed to high rates of transmission. Malaria epidemics, if uncontrolled, follow a natural course. The epidemic grows in a series of steps representative of the incubation interval (the period between the occurrence of infective gametocytes in the primary case and their reappearance in a secondary case), which is about 20 days for *P. vivax* and 35 days for *P. falciparum*. The length of the incubation interval and the degree of the reproduction rate determine the rate of multiplication of transmission, which is much faster in *P.vivax* epidemics than in *P. falciparum* epidemics. In areas where both *P. vivax* and *P. falciparum* are present, the initial stages of an epidemic will be determined by a predominance of *P. vivax* infections and a very gradual increase in severity of the epidemic, while in later stages *P. falciparum* is likely to be abundant. The peak of new infections due to *P. falciparum* epidemics will only be reached when some 50% of the population at risk is infected, unless climatic changes (notably colder temperatures) prevent further transmission.

Potential epidemic situations can often be identified through knowledge of the epidemiology of malaria in a given area combined with an awareness of socio-economic trends. Many epidemic prone situations will, by their nature, stretch across national boundaries. Over the last years, epidemics occurred in countries such as Botswana, Ethiopia, Turkey, Uganda and Vietnam, and in various refugee populations. Control of malaria epidemics involves relieving the immediate clinical consequences, preventing the progress of the epidemic in time and space, and preventing future recurrences. This means improving disease management and providing some form of transmission control. Control of epidemics and reduction of mortality become difficult as epidemics tend to occur in less accessible areas without regular health services.

Historical evolution of malaria distribution[3, 4, 6, 7]

Ancient records suggest that a disease similar to malaria was long known. References to seasonal and intermittent fevers exist in ancient Assyrian, Chinese, Egyptian, and Indian religious and medical texts. Whether they actually describe malaria remains uncertain. Hippocrates, in the fifth century B.C., made the link between the appearance of the disease and the season of the year or the places where his patients lived. He described in detail the clinical picture of malaria and some complications of the disease. Well over 2000 years later, in 1880, Laveran, a French army surgeon in Algeria, was the first to see and describe malaria parasites in the red blood cells of man.

Where the malaria parasite originated from has not been generally agreed upon. Bruce-Chwatt (1985) concluded that the disease probably originated in Africa,

where man acquired the infection from primates and spread it to Europe, Mesopotamia, India, and SE Asia. The reverse has also been argued: that the origin of primate malarias was in Asia and that man has been responsible for the spread of the parasites to African and New World primates[5]. To the present date chimpanzees and gorillas remain the natural reservoir for *P. malariae*. How malaria arrived in the New World remains unclear. It is speculated that *P. vivax* and *P. malariae* were brought from SE Asia to S America by pre-Columbian travellers. *P. falciparum* was then introduced much later by Spanish colonizers and African slaves.

Geographical boundaries of malaria transmission are not constant: they have changed over the centuries with changes in global climate, migration of populations to previously uninhabited areas, and the mode of life. The distribution of malaria within the geographical boundaries of transmission is similarly changing.

Malariological surveys carried out around the turn of the century suggest that malaria at that time probably approached its broadest range of distribution (Map 1). The disease was recorded as high as 2850m above sea level in Tajikistan, and as low as 400m below sea level in the Dead Sea area. At that time, the impact of anti-malarial activities and social and economic development was still limited, and the boundaries of malaria distribution were determined by natural factors relating to the propagation of *Anopheles* mosquitos and the malaria parasites in them. *Anopheles* was absent in Antarctic, parts of Arctic region, and higher mountain ranges, due to unfavourable conditions for their propagation. Natural barriers prevented the spread of *Anopheles* to Pacific Ocean Islands such as New Zealand, American Samoa, Tonga, Cook, Guam, Fiji and New Caledonia. Indigenous malaria was also absent from areas where, although *Anopheles* was present, the average ambient temperature was too low for the parasite to complete the sporogonic cycle, and

Map 1. The original levels of malaria endemicity in the world (Lysenko A. Y., Semashko I. N., 1968, Geography of malaria. In: Lebedew A. W. (ed) Medical Geography. Academy of Sciences USSR, Moscow).

thus for the vector to transmit malaria ("Anophelism without malaria"). Within these broad geographical boundaries the distribution of the malaria problem was very uneven, underlining the focal nature of the disease. The most intensive malaria transmission occurred in areas of tropical Africa and Papua New Guinea where the equatorial climate provided year-round favourable conditions for mosquito survival, man-mosquito contact and completion of sporogony. Marked seasonal variations in malaria intensity were observed in other parts of the (sub-)tropical belt, and an epidemic tendency was recorded in areas towards the edge of the malaria distribution. Malaria was concentrated on the plains, in mountain valleys, and foothills where an abundance of breeding places favoured the propagation of *Anopheles* populations. With increasing altitudes the mosquito fauna became less diverse and mosquito breeding places and densities decreased. This, in combination with a reduction in temperature with altitude, made that malaria ceased to exist at altitudes over 1500m above sea level in most malarious areas. Malaria was also absent in desert areas where conditions for mosquito breeding and survival are particularly difficult. However, it was present in oases where underground water caverns enable mosquito survival.

Both the first and second World War (WWI and WWII) influenced the distribution of malaria. Soon after WWI an extensive epidemic occurred in Russia. Other malaria outbreaks occurred in countries such as England, France, Germany, and Italy. Social upheaval caused by the war combined with favourable climatic conditions to enhance transmission. Between WWI and WWII considerable social and economical development took place in Europe, parts of Asia, and N. America. Changes included improvements in agriculture, housing construction, increased urbanization, and land reclamation notably in Italy and Holland. This, together with wider availability of quinine, resulted in a considerable reduction in the intensity of the malaria problem in Europe and N. America. As a result, by the start of WWII the malaria conditions of Europe and N. America were quite different to that at the time of WWI. Following WWII the spread of malaria was greater, but of lower intensity than the spread which followed WWI. WWII extended well into N. Africa and Asia. Military personnel acquired malaria in these areas and brought *P. vivax*, *P. falciparum* and *P. malariae* back to Europe and America. The difference in intensity of malaria following the two World Wars can be attributed to social and economic changes and to improved chemoprophylaxis and personal and group protection among the troops, particularly after 1944 with the advent of DDT for malaria control. Prior to WWI the spread and intensity of the malaria problem within its boundary of distribution was mainly determined by natural factors. Between the two World Wars, at least in Europe and N. America, this distribution was to a considerable degree determined by the pace of social and economic development and to a lesser extent by organized malaria control operations.

After the end of WWII the situation changed drastically. Residual insecticide spraying was introduced for malaria control, and new synthetic antimalarial drugs became available for treatment. As a result of the selective use of these new powerful tools, together with the progress in socio-economic and health services development, malaria practically disappeared from Western and Central Europe, N. America, Chili, Japan, and from the northern and central parts of the USSR. By the end of the 1950s a population of about 220 million, some 20% of the total population living in malarious areas, had been freed from the risk of the disease. This, together with successes with residual insecticides for malaria control in some countries with (sub-)tropical climates (notably British Guyana, Greece, Italy, Taiwan, and Venezuela), led to the belief that malaria could be eradicated in a relatively short time from most of the malarious areas in the world.

In May 1955, the 8th World Health Assembly decided that WHO should take the initiative in developing an international programme aimed at the worldwide eradication of malaria. The possibility of interrupting malaria transmission with residual insecticides in the most intense malaria foci of tropical Africa was still unproven, and

this part of Africa was temporarily excluded from the global programme. Thus the most severe malaria problems in the world were left unattended, while the more "doable" areas were approached first. The concept of malaria eradication depended heavily on the use of DDT and the availability of chloroquine. The extensive vertical eradication programmes used a "blanket" approach, oblivious to the focal nature of the epidemiology of malaria disease. The global malaria eradication programme, an effort of unprecedented magnitude, attracted over 200 million US$ in international assistance during the ensuing 10 years.

By 1968, as a result of the intensive international efforts, the geographic area of malaria between its northernmost and southernmost boundaries was considerably narrowed, and within these boundaries the distribution and intensity of the malaria problem were greatly reduced. Malaria was eradicated from Europe, the USSR, Australia, Tunisia, Libya, Lebanon, Jordan, Israel, the Korean Peninsula, from all islands with a sub-tropical climate and from many islands with a tropical climate, including the Caribbean islands (except Haiti), Mauritius, Reunion, Taiwan, etc., and from most of the malarious areas of Argentina and South Africa. *P. falciparum* was the first species to disappear from the former fringe areas of the geographic range and from many areas or countries with a sub-tropical climate (N. Mexico, Argentina, S.Brazil, Algeria, Morocco, Turkey, Syria, Iraq, N. Iran, Afghanistan, Nepal, N. China, Korean Peninsula, N. Vietnam). In many tropical countries the proportion of *P. falciparum* infections was markedly decreased, to the level where *P. vivax* became the dominant species. Only in areas where malaria was not amenable to control with residual insecticides, due to exophily of vector populations, poor housing and/or intensive population movements, *P. falciparum* remained the predominant species. *P. malariae* usually fell into the same pattern of disappearance as *P. falciparum*, though occasional indigenous *P. malariae* cases continued to be reported even from areas where malaria was eradicated long ago. With some exceptions (Amazonian Region of Brazil, Colombia, Haiti, Surinam, and some areas of Laos, S. Vietnam, Cambodia, Myanmar and Thailand), *P. vivax* became the major species. In tropical Africa the malaria distribution and prevalence remained practically unchanged with the majority of malaria cases being due to *P. falciparum*.

In the early seventies the concept of malaria eradication had to be abandoned for various reasons, including technical problems, as parasites became increasingly resistant to drugs, and mosquitos resistant to insecticides. Besides, the logistics and resources involved in eradication programmes were too heavy a drain for many national health budgets. When the World Health Assembly in 1969 decided to re-examine the malaria eradication strategy, donors quickly responded with a withdrawal of funding from the programme. Many national programmes in developing countries had already exhausted their capabilities to improve the situation further or maintain the malaria-free status. The economic crisis at the beginning of the 1970s, which heavily affected the developing countries, led to another considerable reduction in antimalarial activities. Prices of insecticides and drugs became unaffordable, even more so where DDT resistance and chloroquine resistance necessitated the purchase of more expensive alternatives. As a result, malaria started a gradual and in some instances dramatic comeback in many Asian and Latin American countries from where malaria had not been completely eradicated. The majority of "post-eradication" malaria epidemics were due to *P. vivax*. However, within a short period of time after the re-establishment of malaria transmission *P. falciparum* usually reappeared, with the exception of countries in Asia Minor. Recent years have seen an intensification of malaria transmission in many previously less affected or unpopulated areas: agricultural development areas, jungle areas with open mining, industrial and agricultural development projects, peri-urban settlements of explosive population growth, areas with socio-political disturbances, civil wars and refugee movements, and so forth. In most of these areas *P. falciparum* has become the predominant species. The northern and southern limits of *P. vivax* and *P.*

falciparum distributions are now almost identical. *P. malariae* has a very patchy global distribution, and is common along the equatorial belt in Africa with relative prevalences as high as 25%. *P. ovale* is restricted to tropical Africa, with rare cases recorded from countries in SE Asia and the Pacific. Its relative prevalence is generally low (between 0.1 and 10%), but may be somewhat higher in forest and coastal regions. The relative prevalence of malaria species is fairly stable through the year in areas with perennial transmission, but may vary in areas with seasonal transmission[6]. Thus the distribution and intensity of the malaria problem are currently governed by natural factors, by the pace and direction of social and economic development including the health sector, and by a country's capability to organize and sustain epidemiologically sound malaria control activities.

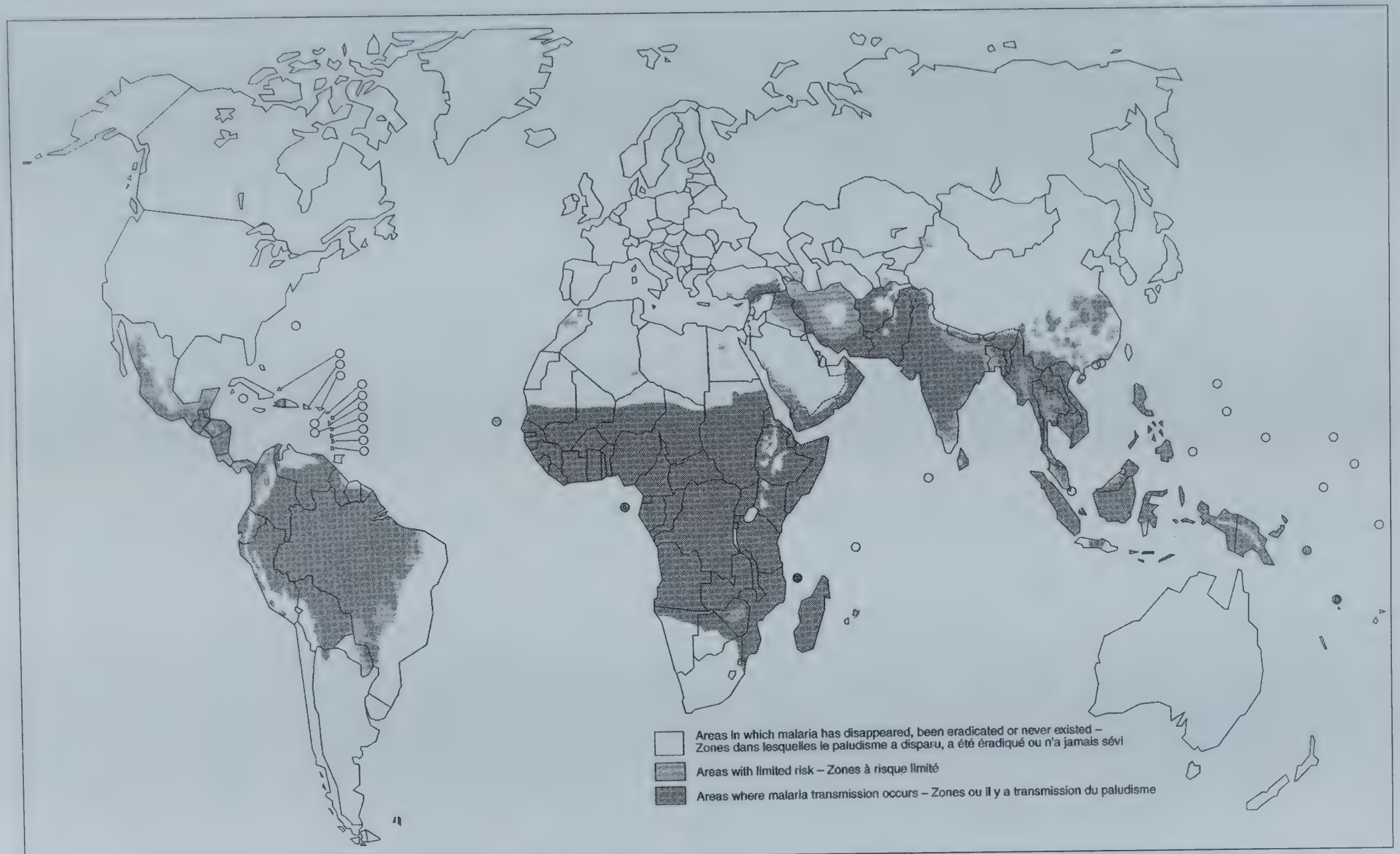
In 1945, only 30% of the world's population was estimated to live in areas free from malaria transmission. By 1959, just after the start of the world-wide malaria eradication campaign, this percentage had increased to 43%. In 1968, at the height of the malaria eradication campaign, 53% of the world's population was free from malaria risk. By 1982 this percentage had gone down again to 44%. In 1993, mainly on account of the eradication of malaria from the most populated areas of China, 64% of the world's population was estimated to live in areas free from malaria transmission. Obviously, since the world population has been growing from 2.9 billion in 1959 to 5.5 billion in 1993, the absolute number of people exposed to malaria risk is still a staggering 2 billion, compared to 1.7 billion in 1959.

Several international regulations remain to help contain the spread of malaria and its vectors. These include the disinfection of aircraft before departure or in transit; disinfection of aircraft, ships and other vehicles on arrival if the health authority at the place of arrival has reason to suspect importation of malaria vectors; and the enforcement and maintaining of rigid antimosquito sanitation within the mosquito flight range of all ports and airports. In special circumstances antimalarial drugs may be administered to potentially infected migrants, refugees, seasonal workers, and persons taking part in periodic mass movement into areas or countries where malaria has been eliminated[7].

Present geographic distribution of malaria problem (Map 2)[8]

In *Africa south of the Sahara* malaria remains one of the worst public health problems. It is estimated that 270 to 480 million clinical malaria cases occur annually, some 140 to 280 million of them occurring in children under 5 years old. Only a fraction of malaria cases are reported. More than 90% of the population in the WHO African Region live in areas where they are at risk of getting malaria, with 74% of the population living in highly endemic areas where malaria transmission is intense and perennial (equatorial and tropical forests and Sudanese savannas at altitudes up to 1000 m with an average rainfall over 2000 mm/year). At altitudes over 1500 m and rainfall below 1000mm/year, endemicity decreases and the potential for epidemic outbreaks increases. *P. falciparum* is the predominant species in the highly endemic areas, where some 30% of febrile illnesses are attributable to malaria. Mortality is concentrated in the younger age groups. Among children referred to hospitals with severe malaria, case fatality rates of 10-30% have been reported. In rural areas with little access to adequate treatment these rates might be even higher. Overall, malaria produces considerable impact on the health of young African children, leaving neurological sequelae, increasing susceptibility to other infections, and hampering development.

In the WHO *Eastern Mediterranean* Region, a group of countries stretching from Morocco to Pakistan, about 45% of the population live in areas where they are at risk of *P. falciparum* and *P. vivax* transmission. Another 15% is at risk of *P. vivax* alone. It is estimated that some 13 million cases of malaria occur annually in the region, the large majority caused by *P. falciparum*. Some 35,000 deaths are estimated attributable to malaria, occurring chiefly in Sudan, Somalia and Yemen. Over 95% of malaria cases occur in just five countries:


Areas in which malaria has disappeared, been eradicated or never existed –
Zones dans lesquelles le paludisme a disparu, a été éradiqué ou n'a jamais sévi
Areas with limited risk – Zones à risque limité
Areas where malaria transmission occurs – Zones ou il y a transmission du paludisme

Map 2. World malaria situation 1993. The designation employed and the presentation of material on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines represent approximate border lines which there may not yet be full agreement (© World Health Organization 1995 - Organisation Mondiale de la Santé 1995.

Sudan, Somalia, Yemen, Afghanistan and Iraq. In these countries, as well as in Djibouti, widespread poorly controlled malaria is due to extremely favourable conditions for transmission, and/or disruption of control programmes due to war and political instability. In the remainder of countries malaria is under more or less firm control or has been interrupted altogether. In Iraq, Morocco and Syria *P. falciparum* has been selectively eradicated, with *P. vivax* transmission remaining[9].

Malaria transmission is a public health problem in 21 countries and territories in *the Americas*. During 1993, a total of almost a million microscopically confirmed malaria cases (*P. falciparum* and *P. vivax*) were reported, a slight decrease compared to previous years. However, records of the actual number of malaria treatments administered through the health services indicate incidences some five times higher. Taking into account that the private sector and self-treatments are not included in these data, officially confirmed malaria cases appear to be only a small portion of the actual number of patients with malarial disease. Nearly half of the cases were registered in Brazil; a third originated from the Andean countries (Bolivia, Colombia, Ecuador, Peru, Venezuela) and almost one sixth from Central America and Mexico. While the overall proportion of registered *P.falciparum* infections declined slightly to some 30% in 1993, their proportion increased in Bolivia, Ecuador, French Guiana, Guatemala, Mexico, Peru and Venezuela. Almost two-thirds of all falciparum infections detected in the Americas occurred in Brazil.

During the last 3 years, the overall number of malaria cases reported in the WHO *South East Asia* Region remained stable at 2.6 to 2.7 million cases annually. India represents some 80% of these recorded cases. The real malaria incidence may be about 6-7 times higher, representing between 16 and 19 million malaria cases annually, of which more than half are falciparum malaria. Most of the malarious areas, accounting for nearly half of the total number of cases, are situated in forests, forest-fringe areas, forested hills, development project areas and their surroundings. Due to the exploitation of natural resources forests become more accessible and movements of populations with low immunity into such areas result in malaria epidemics. Many such areas are close to the international borders. Their population is very mobile and the peripheral health structure is lacking and inadequate for the early diagnosis and treatment of malaria. The world's worst multi-drug resistance problems occur in such areas, especially in border areas between Thailand and Myanmar and Thailand and Cambodia. Some areas in South East Asia rely for malaria control on extensive vector control operations, leaving the malaria situation precariously influenced by the quality and extent of such operations and the development of insecticide resistance.

The total malaria incidence of *East Asia and the Western Pacific* declined by some 20% between 1992 and 1993. Australia, Brunei Darussalam, the Korean Peninsula, Hong Kong, Japan, Macao, Mongolia, Singapore, large areas of China and most of Oceania remained free of malaria altogether. The majority of cases were reported in Vietnam, the Solomon Islands, Myanmar, and Thailand. Several countries experience severe and deteriorating falciparum malaria problems, especially in peripheral parts of the country, often combined with the occurrence of multidrug-resistant *P. falciparum*. Malaria is for instance a major cause of illness and death in Cambodia, Laos and Vietnam. 99.000 confirmed malaria cases and 1100 deaths due to malaria were recorded in Cambodia in 1993, but real figures are estimated to be over five times as high: some 0.6 million clinical cases and 5000 to 10.000 death annually. In Laos an estimated 14.000 deaths due to malaria occur each year. Vietnam has an estimated number of clinical cases in the order of 1.5 million per year. Levels of antimalarial drug resistance in some parts of these countries are so high that chloroquine and sulfadoxine-pyrimethamine are useless against falciparum malaria. In addition, there is localized resistance to both quinine and mefloquine. In some provinces the main treatment for falciparum malaria is now artemisinin or artesunate. Malaria is holoendemic in parts of Papua New Guinea, very similar to parts of Africa. Van-

uatu and the Solomon Islands have the highest overall malaria morbidity of this region with 66 and 364 malaria cases per 1000 population respectively, some 60% due to *P. falciparum*.

Europe experiences a steady influx of malaria cases imported from tropical countries. A substantial portion of these occur in nationals from endemic countries, who upon living in Europe for some time, and thereby losing their immunity, subsequently become infected when visiting their relatives back home. Isolated cases of local transmission in Europe (excluding Turkey) occur rarely, including some cases of "airport malaria". In Turkey, malaria incidence has increased over five-fold since 1990. The provinces most affected are located in south-east Anatolia, most of them bordering Iraq. In this area of extensive population movements one of the largest development projects in the Middle East is under way, including some 22 new dams for electricity production and irrigation.

Present epidemiology of drug-resistant malaria

P. falciparum. Strains of *P. falciparum* resistant to chloroquine were first reported in S America and SE Asia in the early 1960s. Since then they have swept across the globe. Among the countries where falciparum malaria is endemic, only those of Central America have as yet not recorded chloroquine resistance. Chloroquine resistant *P. falciparum* spread over almost all of tropical Africa in the 1980's. In many African countries, especially in eastern Africa, high levels of resistance pose increasing problems for the provision of adequate treatment. Surveillance has indicated that the development of resistance to chloroquine has been accompanied by an increasing incidence of severe malaria. An increase in the prevalence of anaemia in very young children may be one of the early signs of chloroquine failure. Resistance to sulfadoxine/pyrimethamine is widespread in South-East Asia and South America but focal and uncommon in other parts of the world. In certain border areas of Thailand over 50% of falciparum infections no longer respond to mefloquine therapy. Reduced sensitivity of *P. falciparum* to mefloquine has only rarely been reported in *in vivo* studies in Africa. It has not been reported from the Americas. In several countries of South-East Asia as well as in Brazil quinine plus tetracycline is used for treatment for uncomplicated malaria, and sensitivity to quinine is diminishing. Consequently, artemisinin and its derivatives are increasingly being deployed for first-line treatment in these areas.

P. vivax. Resistance of vivax malaria strains to chloroquine, first documented in 1989 in infections from Papua New Guinea, has now been confirmed in Indonesia, Myanmar and Vanuatu. In some localized foci in Indonesia and Papua New Guinea, 20-30% of patient infected with vivax malaria suffer recurrences of parasitaemia 1 to 3 weeks after a course of 25 mg chloroquine base/kg. Elsewhere chloroquine resistance does not yet pose an operational problem.

P. ovale and *P. malariae*. Occasional reports of drug resistance exist, as yet without operational significance.

World maps depicting the geographic distribution of drug-resistant *P. falciparum* are somewhat misleading. The mere reporting of drug resistance does not imply that all parasite strains in an entire country show resistance; chloroquine may well be operationally effective treatment for the large majority of malaria cases. Similarly, the absence of reported drug resistant *P. falciparum* may only point to the absence of a regular monitoring system. However, on a country basis the accurate monitoring and mapping of *in vivo* drug resistance is an important, essential part of the national malarial drug policy.

Epidemiology in practice

a) Arm chair epidemiology of local malaria situations[10]

On the basis of easily recognizable ecological and social characteristics, most malaria situations in the world can be identified as falling within a few major epi-

demiological types. For each of these, certain risks are particularly important, and certain approaches to control are more likely to succeed than others. Planning malaria control for a given area starts with identification of the major epidemiological types within it, followed by collection of information on the particular risks and on control approaches that might be realistic (see Table 1).

b) High risk of malaria disease and death

To get an overview of the malaria problem in any given area, and to target control activities towards a reduction in morbidity and mortality, it is important to realize who are most likely to be affected by the disease, and who are most likely to die of it. The individual risk of serious illness and death is closely related to the absence of acquired immunity. Immunity to malaria is only partial and for a duration that is a function of the intensity and frequency of prior infections. In areas with seasonal or epidemic malaria where disease is infrequent, adequate protective immunity may never build up and the disease will affect all age groups. This will also be the case in previously endemic areas that have become epidemic, for instance as a result of increasing urbanization. Even "fully immune" adults who are no longer exposed to infection lose their immunity over 1-2 years.

High risk population groups. The vast number of malaria deaths occur in endemic areas of tropical Africa among children aged 6 months to 3 years, especially in remote rural areas with poor access to health services. In these areas falciparum malaria may kill up to 5% of children before the age of five years. The surviving children who continue to be exposed to infection gradually acquire partial immunity. Malaria is a major cause of anaemia in children, and increases their vulnerability to other diseases. It is estimated that malaria kills about 1 million children below the age of 5 every year. For some of them malaria will not be the only cause of death. Another group at high risk of malaria are pregnant women. In the majority of developing countries these will be young women in their teenage years. They are at higher risk of clinical disease, and at higher risk of severe disease. In endemic areas malaria substantially increases the risk of maternal anaemia, abortion, stillbirth, prematurity and low birth weight during a woman's first pregnancy. With subsequent pregnancies this risk diminishes. The risks of malaria infection in non-immune pregnant women include spontaneous abortion in up to 60% of cases and a maternal mortality rate of up to 10%[11]. Other high risk groups include non-immunes such as travellers, refugees, displaced persons, or labour forces entering into endemic areas.

High risk occupations. In some areas, including parts of SE Asia and S America, malaria transmission may be geographically linked to specific activities and occupations, such as wood cutting or gem mining in forest areas. In these areas the disease affects specific segments of the population. Low immunity combined with inappropriate and delayed treatment may lead to high mortality and severe morbidity in those affected. In addition, some of the world's worst antimalarial drug resistance problems occur in these areas.

High risk environment. Persons who normally should not be at risk of serious life-threatening disease are put in danger when they have no access to early diagnosis and adequate treatment of malaria disease. This can be due to inadequate health infrastructure, inadequately trained health staff, or scarcity of resources (of the patient and/or health services). Socio-political unrest, population movements and civil war may make health facilities unaccessible or non-functional.

c) Fine tuned management information needs for malaria control

Assessment and analysis of local malaria problems are a prerequisite for targeted control activities. An appropriate epidemiological information system is thus an essential part of a control programme. The Global Strategy on Malaria Control recommends that epidemiological information should include morbidity and mortality data, as well as information on underlying factors relating to the human population,

Malaria type (main occurence)	Characteristics		Actions required	
	Epidemiological	Operational	Disease management	Prevention
Savanna malaria	• Perennial transmission, with seasonal variations away from equator. *Plasmodium falciparum* is overwhelmingly predominant. • Morbidity and mortality mainly in young children and pregnant women. • Expansion of drug resistance.	• Insufficient coverage by health services. • Malaria control programmes most often rudimentary.	• Expansion through formal and informal general health services. • Strengthening of capacity for management of complicated malaria and treatment failures in health services.	• Investigations on potential role of impregnated bednets and curtains. • Chemoprophylaxis for pregnant women, unless precluded by drug resistance.
Malaria of plains and valleys outside Africa (Central America, China Indian subcontinent)	• Variable, mainly moderate transmission. • *P. vivax* may predominate. • Strong seasonal variation. • Risk of epidemics. • Drug resistance generally well established.	• Large-scale insecticide spraying programmes often ineffective. • Inadequate disease management. • Insufficient general health services and private services in some areas.	• Responsibility for disease management to be assigned to general health services. • Establishment/strengthening of epidemiological information systems.	• Reorientation for better targeting of vector control. • Environmental management in some areas. • Use of impregnated bednets (proved useful in China).
Highland and desert-fringe malaria (African and South-East Asian highlands, Sahel, southern Africa, South-West Pacific)	• Risk of epidemics due to climatic aberrations, changing agricultural practices or migration to malarious areas.	• Presence of health services variable. • Preparedness for management of malaria cases may be poor in habitually malaria-free areas. • Terrain, distances and precipitation present obstacles to malaria control.	• Facilities to be established rapidly with effective drugs in case of outbreak. • Active detection and treatment of fever cases may be justified. • Health services must be aware of risk of outbreak.	• Insecticide spraying can often rapidly curb transmission and sometimes restore malaria-free status.
Agricultural development projects (Africa, Asia, South America)	• Increased transmission due to irrigation in certain circumstances. • Risk of seasonal malaria outbreaks due to attraction of non-immune labourers.	• Insecticide resistance frequent in cotton-growing areas. • Some financial resources are available for malaria control.	• Services for early treatment to be established/strengthened.	• Environmental management to be considered at the planning stage. • Appropriate siting and screening of dwellings. • Use of impregnated bednets. • Introduction of larvivorous fish in certain rice-growing areas. • House-spraying or chemoprophylaxis if needed.
Urban and periurban malaria (Africa, South America, South Asia)	• Transmission and population immunity highly variable over short distances. • Epidemics caused by specially adapted vectors in South Asia.	• Relatively good coverage by health services. • Variety of antimalaria drugs available from different sources. • High human population density. • Breeding sites identifiable.	• Standardization or harmonization of treatment practices.	• Larval control; in some situations chemical larviciding is preferable. • Personal protection.

Table 1. Characteristics of the major epidemiological types of malaria actions required to improve malaria control (Source: A Global Strategy for Malaria Control, WHO, 1993).

the parasite, the vector and the ecosystem, and on the impact of malaria. In addition, the status of health services and of existing vector control activities should be taken into account in planning, as should the constraints and opportunities posed by resources and the physical and administrative infrastructure.

d) The bare epidemiological necessities

For district health officers, the bare necessity will be to have descriptive information on "person, place and time" relating to malaria morbidity and mortality. In short: WHO, WHERE, WHEN, HOW MUCH and WHAT KIND OF? The district officer needs to know who gets affected (age, sex, occupation), which villages or areas are most affected, and during which times of the year. To target effective first line treatment with antimalarial drugs, the patterns of different malaria species present in the district must be known (*P. falciparum* or other species), and their patterns of response to treatment. For the latter, WHO has developed a simplified *in vivo* monitoring system, based on the active follow-up of malaria patients for signs of drug failure[12]. In the absence of such *in vivo* monitoring, treatment failures reported by health centres will give a rough and ready indication of the effectiveness of first line antimalarial drugs.

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IMMUNOLOGY OF MALARIA

M. Hommel

Introduction

The protective effects of immunity on malarial infection in an individual living in a malaria-endemic area may be summarised as follows. During infancy, protection is provided during the first 6 months of life by the passive transfer of maternal immunity; as this wanes, there is a progressive increase of acquired immunity with age but a number of years of exposure are required before immunity has any measurable effect on the intensity of infection. There is a period during childhood when the intensity of clinical paroxysms are substantially reduced, while there is still little or no effect on the incidence of parasite infection; this state of "*anti-disease*" immunity may precede by a number of years the occurrence of "*anti-parasite*" immunity. The first signs of anti-parasite immunity are a reduced parasite density and a shortening of patent parasitaemic episodes and this is eventually followed by a reduction in the number of parasitaemic episodes observed. Once it is acquired, this state of immunity will be maintained for life or at least as long as the individual remains continuously in the endemic area; this immunity is never complete (usually referred to as "*semi-immunity*") and, throughout adult life, parasitaemic episodes may be observed, which are usually of low intensity, short duration and clinically asymptomatic. A dramatic breakdown of this state of immunity appears to occur during pregnancy, particularly in primigravidae.

This paper will describe what is known of the development of immunity to malaria and discuss the relevance of immunity to our understanding of malaria pathophysiology, diagnosis and control.

Evidence of malaria immunity

Epidemiological studies have provided the outline of how malaria immunity is acquired in man and Christophers, as early as 1924, was able to give a description of the features and stages of acquisition of immunity[1], which is broadly the picture that was later confirmed by careful longitudinal studies performed in The Gambia and in Nigeria[2,3].

It was the detailed studies of induced malaria in neurosyphilitic patients ("malariotherapy") which produced evidence that immunity was *species-specific* (e.g. no cross-protection between *Plasmodium vivax* and *P. falciparum*) and to a large extent *strain-specific*[4]. These studies also indicated that immunity to *P. falciparum* was acquired more slowly than that to *P. vivax* or *P. malariae* and that, once present, immunity could persist for several years.

The use of experimental models for malaria, particularly in non-human primates and in laboratory rodents, have confirmed these features and have also shown that immunity was *stage-specific* (i.e. there is little or no cross-immunity between different stages of the life-cycle of

malaria parasites). It should be stressed that the immune response of the host to the parasite is a complex series of events, where the relative importance of both host and parasite components may vary considerably from one host-parasite combination to another; most animal models are inadequate in reproducing what happens in man and, while they can help the researcher in exploring specific issues, any extrapolation to human disease or generalisation has to be considered with extreme care. This feature must always be in the back of our mind when attempting to interpret experimental results which may otherwise appear to be contradictory if not entirely confusing; an example of this is the radically different immune response of mice to two murine malaria parasites, *P. yoelii* and *P. chabaudi*, where protection is mostly antibody-mediated in the former and mostly cell-mediated in the latter.

Innate resistance

Some individuals are either naturally resistant to malaria infection or less likely to develop a severe form of the disease. Innate resistance to infection is generally only a partial resistance, and may be linked either to the fact that malaria parasites find it harder to invade certain types of human erythrocytes (e.g. ovalocytic cells are resistant to invasion by all human malaria parasites, while Duffy negative cells are resistant to invasion by *P. vivax* only) or to the fact that certain host erythrocytes have a reduced ability to sustain the growth of parasites (as is the case in individuals with a G-6-PD deficiency or with certain abnormalities of haemoglobins, as sickle cell anaemia or β-thalassemia)[5]. Table 1 provides a list of the different mechanisms of innate resistance to malaria. The preference for certain host cells also explains why *P. vivax* or *P. ovale* only develop in reticulocytes, while *P. falciparum* can develop in erythrocytes of all age. It has also been reported that certain individuals living in holoendemic areas for malaria have no history of detectable malaria infection and this has been explained by the presence of antiparasitic molecules in their serum; for example, such serum factors reported from patients in Sudan have been described as "crisis forming factor", because this material causes intracellular death of malaria parasites in vitro, which is comparable to what happens with the serum of immune individuals at the time of parasite clearance (i.e. "crisis")[6]. Genetic differences in host susceptibility may also affect the individual response to disease or the ability to mount an effective immune response (see below).

Mechanism of resistance	Degree of Resistance	
• Red cell recognition		
lack of glycophorin	+	in vitro
lack of sialic acid	+	in vitro
• Red cell invasion (reduced deformability)		
ovalocytosis	++	in vivo
elliptocytosis	+	in vivo
• Intracellular development		
Haemoglobin S (sickle cell trait)	+	in vivo
G6PD deficiency	+	in vitro
Pyridoxal kinase deficiency	+	in vitro
Thalassemia β	+	in vivo
Foetal haemoglobin	+/–	in vitro
Haemoglobin C, D, E	+/–	in vitro

Table 1. Innate resistance to *falciparum malaria*. List of situations where the host or the host cell was shown to be fully or partially resistant to *Plasmodium falciparum*.

The antigenic structure of malaria parasites

Malaria parasites are lower Eukaryotes, with a genomic complexity which is five times greater than that of bacteria, with a complex life-cycle involving both a mammalian and an invertebrate host, where the parasite has to invade three very different types of cells (erythrocytes, hepatocytes and mosquito midgut epithelial cells) and needs to survive in very different hostile environments. In the circumstances, it is not surprising that the antigenic structure of the parasite will be radically different from one stage of the life cycle to another. This is illustrated in figure 1, which shows the localisation of 40 major antigens of *P. falciparum* in sporozoites, pre-erythrocytic schizonts, merozoites, erythrocytic schizonts, gametocytes and gametes. A precise description of these different antigens would be outside the scope of this paper and the reader may refer to excellent recent reviews by Howard and Pasloke and by Coppel[7, 8]. Many of these antigens have been remarkably well-studied and a complete gene sequence is known for most of them.

Apart from their high degree of stage-specificity, malarial antigens present a number of other notable features:

i) there is a relatively small number of molecules actually expressed on the surface of invasive stages and this reflects the fact that these stages are generally covered

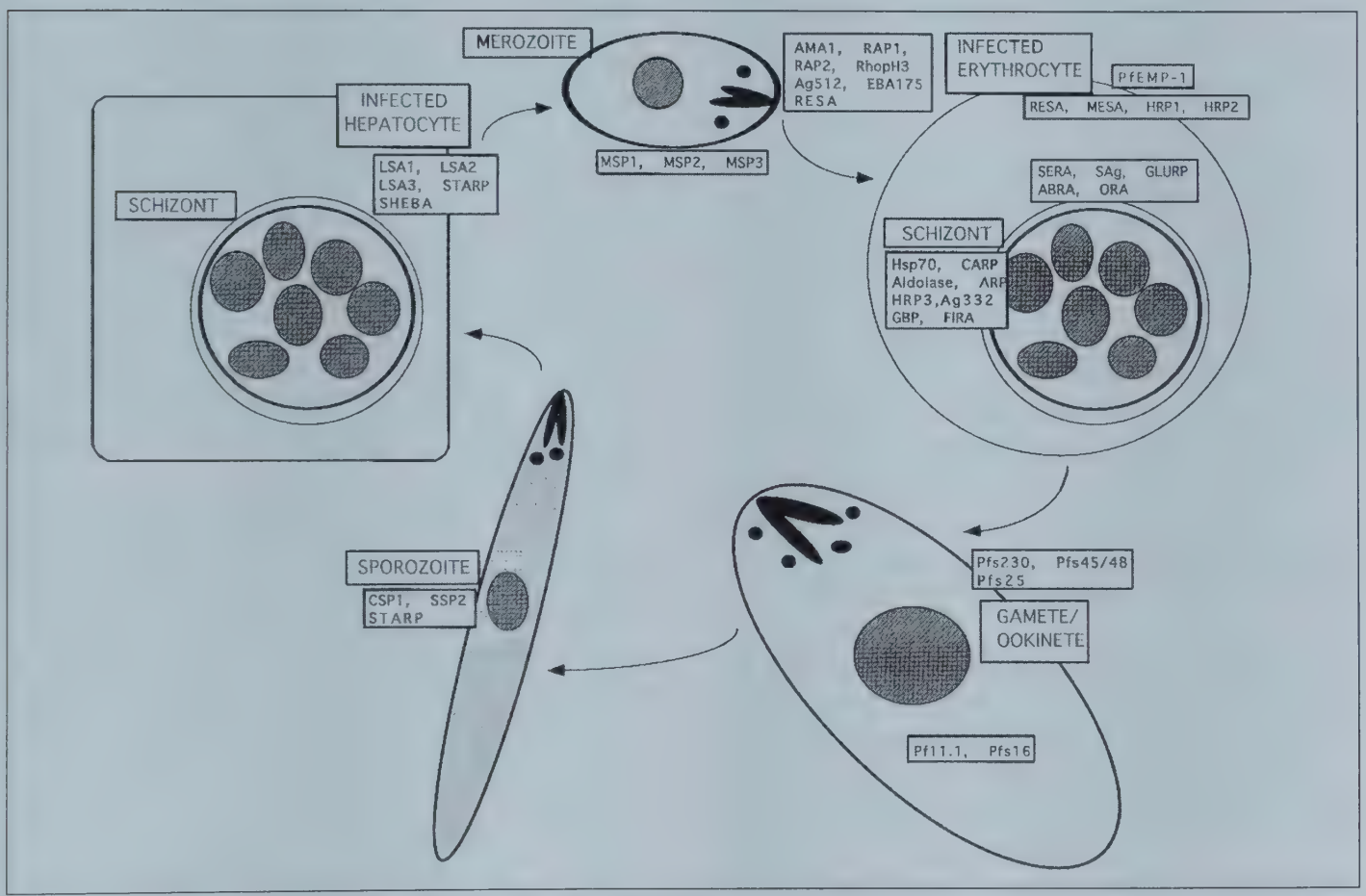

Fig. 1. Simplified life-cycle of *Plasmodium falciparum* showing the important antigens for each stage and their localisation. Abbreviations signify: heat shock protein (hsp70); asparagine-rich protein (ARP); clustered asparagine-rich protein (CARP); ring-infected erythrocyte surface antigen (RESA); mature-parasite infected surface antigen-2 (MESA); histidine-rich protein (HRP); glycophorin-binding protein (GBP); Falciparum interspersed repeat antigen (FIRA); serine-rich antigen (SERA); glutamate-rich protein (GLURP); acidic basic repeat antigen (ABRA); parasitophorous vacuole octapeptide repeat antigen (ORA); erythrocyte membrane-associated malaria protein-1 (PfEMP1); merozoite surface antigen (MSP); apical membrane antigen-1 (AMA1); erythrocyte-binding antigen ABA175); rhoptry-associated protein (RAP); circumsporozoite protein (CSP1); sporozoite surface, infected hepatocyte sporozoite surface protein-2 (SSP-2); sporozoite surface liver-specific antigen (LSA); sporozoite threonine and asparagine-rich protein (STARP).

by a surface coat with crucial biological functions in terms of recognition and adherence to future host cells (e.g. CSP-1 & SSP2/TRAP on sporozoites, MSP-1, MSP-2 & MSP-3 on merozoites, Pfs230, Pfs45/48 & Pfs25 on gametes or öokinetes);

ii) extracellular stages all possess the invasion organelles characteristic of Apicomplexan Protozoa (i.e. apical complex with rhoptries and micronemes) and many immunodominant antigens appear to be associated with these organelles (e.g. AMA-1, RAP-1 & RAP-2, EBA-175, Ag512)[9]. Although these molecules are not actually on the surface of the cells, they seem nevertheless to play an important role at the parasite-host cell interface and immune reactions against them interfere with invasion. It is conceivable that the good immunogenicity of these molecules may reflect more their uniqueness (i.e. no similar antigens in the host) than their true biological importance;

iii) some molecules of parasite origin are expressed at the surface of infected erythrocytes or infected hepatocytes, either directly by the parasite in order to fulfil a fundamental parasite function (as is probably the case for PfEMP-1, expressed on the surface of host erythrocyte for cytoadherence to vascular endothelium) or by means of a host processing function related to MHC Class I expression (as is probably the case for all the exo-erythrocytic antigens at the surface of hepatocytes, including CSP-1, LSA-1 & LSA-2, STARP);

iv) finally, all the other antigens listed on figure 1 (and probably many others not listed) are found inside the parasite and as such would logically not be expected to play a role in protection against malaria. These molecules are either excreted during schizogony (by means of a transfer from the parasite to the parasitophorous vacuole and then to the outside of the cell) or, more often, released at the time of schizont rupture. They may be highly immunogenic and be responsible for a considerable percentage of the total host immune response and consequently be involved in immunopathological events; these molecules may also be of interest for immunodiagnosis.

Antigenic diversity

Beside the marked species- and stage-specificity of immunity to malaria parasites, there are also marked differences between isolates of the same species. This had been observed in early malariotherapy studies, as it proved necessary to change frequently from one isolate to another in order to obtain substantial levels of parasitaemia in a patient after repeated infections. Studies of field isolates of *P. falciparum*, using various methods of analysis (including the study of isoenzymes, two-dimensional gel electrophoresis, monoclonal antibodies, pulse-field electrophoresis of chromosomes or restriction fragment length polymorphism), have provided further evidence of the truly considerable diversity of the phenotype and genotype of field isolates.

The extent of diversity varies from one antigen to another and several types of polymorphisms have been described. The differences between different alleles of a given gene represent a relatively restricted form of polymorphism (as is observed, for example, for the different alleles of MSP-1). In contrast, a much more extensive polymorphism may be generated by variation of a repeat domain in the molecule (as was described for molecules like S-antigens, Pf11.1 or FIRA). The antigens expressed on the surface of infected erythrocytes (e.g. PfEMP-1) appear to be particularly polymorphic.

The considerable diversity between field isolates may be one of the major reason why a long time is required to develop immunity, since an individual living in an endemic area would need to be exposed to a vast repertoire of local strains[10]. However, before accepting that antigenic diversity is a likely explanation for the slow development of protection, it is necessary to assume that strain-specific immunity can ultimately be transcended by a broader species-specific immunity (the nature of which is as yet unknown); in the absence of such a transcending immunity, it would be difficult to understand why infections become rarer in adults since adults continue to be exposed to new isolates.

Antigenic variation

Malaria parasites, as many other micro-

organisms, are capable of changing periodically the expression of their antigens; this provides the parasite with a powerful means for adapting to changing environments in the host and also for evading host immunity[11]. In malaria, antigenic variation was first described in the *P. knowlesi*/rhesus monkey model, but it has since been shown to exist also in *P. chabaudi*, *P. falciparum* and *P. fragile*. Although antigenic variation has not yet been demonstrated for *P. malariae* or *P. vivax*, the long chronicity of the former and the presence of successive waves of recrudescence in the latter are suggestive.

Much of the studies on antigenic variation have concentrated on the molecules expressed on the surface of infected erythrocytes (particularly PfEMP-1 in *P. falciparum*); as many as 10 different variant surface antigens have been isolated from a single parasite strain sub inoculated in squirrel monkeys[12]. The rate of switching from one set of antigens to another is thought to be very fast; in a study of *P. chabaudi* in mice, 40% of the parasites present at the peak of infection were already different from the original inoculum, while in an *in vitro* study of *P. falciparum* it was estimated that 2% of parasites switched to a new antigen in every erythrocytic cycle[13]. Antigenic variation of *P. falciparum* has recently been linked to a large gene family (the *var* genes), which appears to be differentially expressed in different individual parasites[14-15]; it has been calculated that the genome of a given clone of *P. falciparum* has between 50 and 150 different *var* genes (i.e. as much as 6% of the total genome) and all existing data suggest that only one *var* gene may be expressed at the time in a given parasite. Other malarial antigens, beside PfEMP-1, may also have the ability to switch to alternative gene expression, particularly when submitted to a strong selective pressure, but these have not been extensively studied so far.

Mechanisms of anti-parasite immunity

Potential effector mechanisms of immunity include antibodies, macrophages, cytotoxic T cells and a variety of soluble mediators and cytokines. The respective role of these different effector mechanisms on the survival of malaria parasites is variable from one stage in the life-cycle to another (e.g. predominantly antibody-mediated mechanisms against erythrocytic stages and predominantly cell-mediated against pre-erythrocytic stages), but there is probably no situation where a single effector mechanism can be solely incriminated. The sad reality is that our understanding of how natural acquired immunity operates is still very incomplete.

Antibodies

It is well-established that antibodies can protect against malaria and the strongest arguments to support this view have been derived from passive transfer experiments where a naive recipient host with malaria is given immune immunoglobulin and is shown to clear its infection. While the classic experimental transfer of immunoglobulins from adult Gambians to infected children is frequently quoted[16], equally convincing evidence is provided by the observation of a strong protective immunity during the first few months of life in infants in endemic areas, who have received IgG's from their immune mother. Other supportive evidence comes from a large number of experimental studies in animal models using adoptive transfer of immune B-cells or showing that infection in B-cell deficient animals have often a fatal outcome.

Antibodies may interfere with parasite development at different sites: i) by blockage of merozoite dispersion; ii) by inhibition of invasion (whether of merozoites into red cells or of sporozoites into hepatocytes); iii) by intracellular killing of erythrocytic stages (presumably by a form of metabolic poisoning induced by interaction of antibodies with specific "pumps" introduced by the parasite into the red cell membrane); or, iv) by the inhibition or reversal of cytoadherence, which obliges the infected erythrocyte to undergo 20 times every hour the perilous journey around the peripheral bloodstream of the host, including passage through the spleen, instead of remaining safely sequestered in capillary venules. Antibodies to sexual stages of the parasite may be

picked-up, together with gametocytes, by a feeding mosquito and thus block gamete fecundation, a feature which forms the rational basis for the development of transmission-blocking immunity. Finally, antibodies may act in co-operation with various cells (incl. macrophages and T cells) to increase cell-mediated killing.

Cell-mediated responses

Evidence from animal models (for example, experiments in T-cell deficient animals, passive transfer studies or the deletion of specific subsets of T cells) have suggested that T lymphocytes are required for the control of infection by malarial parasites and that they are essential for protection against re-infection (for details regarding such experiments, the reader is referred to excellent review by Troye-Blomberg and Perlmann)[17]. The pre-erythrocytic stages are the most likely targets for cytotoxic T lymphocytes (CTL), since infected hepatocytes are the only stage in the malarial life-cycle expressing MHC Class I molecules; in contrast, sporozoites or merozoites are likely to be poor targets for cell-mediated immunity, and it is not known whether infected erythrocytes are susceptible to be killed by T cells (a suggestion that killing may occur by ADCC involving K cells and antibody has never been confirmed). In contrast, phagocytosis by macrophages is a particularly important mechanism for removing infected erythrocytes (e.g. when crossing the spleen) and this effector function may be amplified both by macrophage activation or by co-operation with cytophilic antibodies; a similar mechanism may also operate to remove merozoite or sporozoite (particularly since the latter has to have close contact with Kuppfer cells before entering the hepatocyte).

Soluble mediators and cytokines

A number of cytokines have been shown to be produced in increased amounts during malaria and some of these are capable of interfering with the development of malaria parasites at different stages of their life-cycle. These include cytokines produced by T cells (e.g. IFN-γ, TNF-α, GM-CSF, IL-4, IL-6, IL-8, IL-10 and lymphotoxin), but also cytokines produced by other cells (e.g. TNF-α or IL-1)(this topic has been extensively reviewed by Kumaratilake and Ferrante)[18]. These cytokines may act directly on infected cells (as does TNF), but more often they act indirectly by inducing the production of soluble mediators by activated macrophages or neutrophils (for example, generation of oxygen-derived reactive species, ODRS, or production of nitric oxide, NO); a cascade of reactions leading to the production of NO is probably responsible for the killing of infected hepatocytes, while both ODRS and NO may be involved in the production of "crisis forms" in infected erythrocytes.

Mechanisms of anti-disease immunity

The concept of malaria as a toxic disease (with similarities with bacterial endotoxaemia) is not new, since a good description of this was given by Sinton in 1939[19]; what is new is the description of a cascade of events involving a series of "malaria toxins" which act on host cells, which in turn produce a variety of molecules responsible for many of the symptoms observed during a malaria paroxysm (incl. fever, headaque, anaemia, hypoglycaemia and gastro-intestinal symptoms). TNF-α appears to be the central molecule in this cascade and, in order to understand current concepts regarding anti-disease immunity, it is necessary to examine the mechanisms for TNF induction, the effects of TNF on the host and how immunity may interfere with these. These various mechanisms are summarised on figure 2.

Induction of TNF

TNF is induced as the result of malarial antigens being released into the peripheral blood at the time of schizont rupture; this occurs by means of three different pathways:

i) the effect of malarial antigens on TH1 $CD4^+$ cells, which in turn produce IFN-γ (and some TNF), which acts on macrophages to produce inflammatory cytokines (incl. TNF and IL-1). This pathway

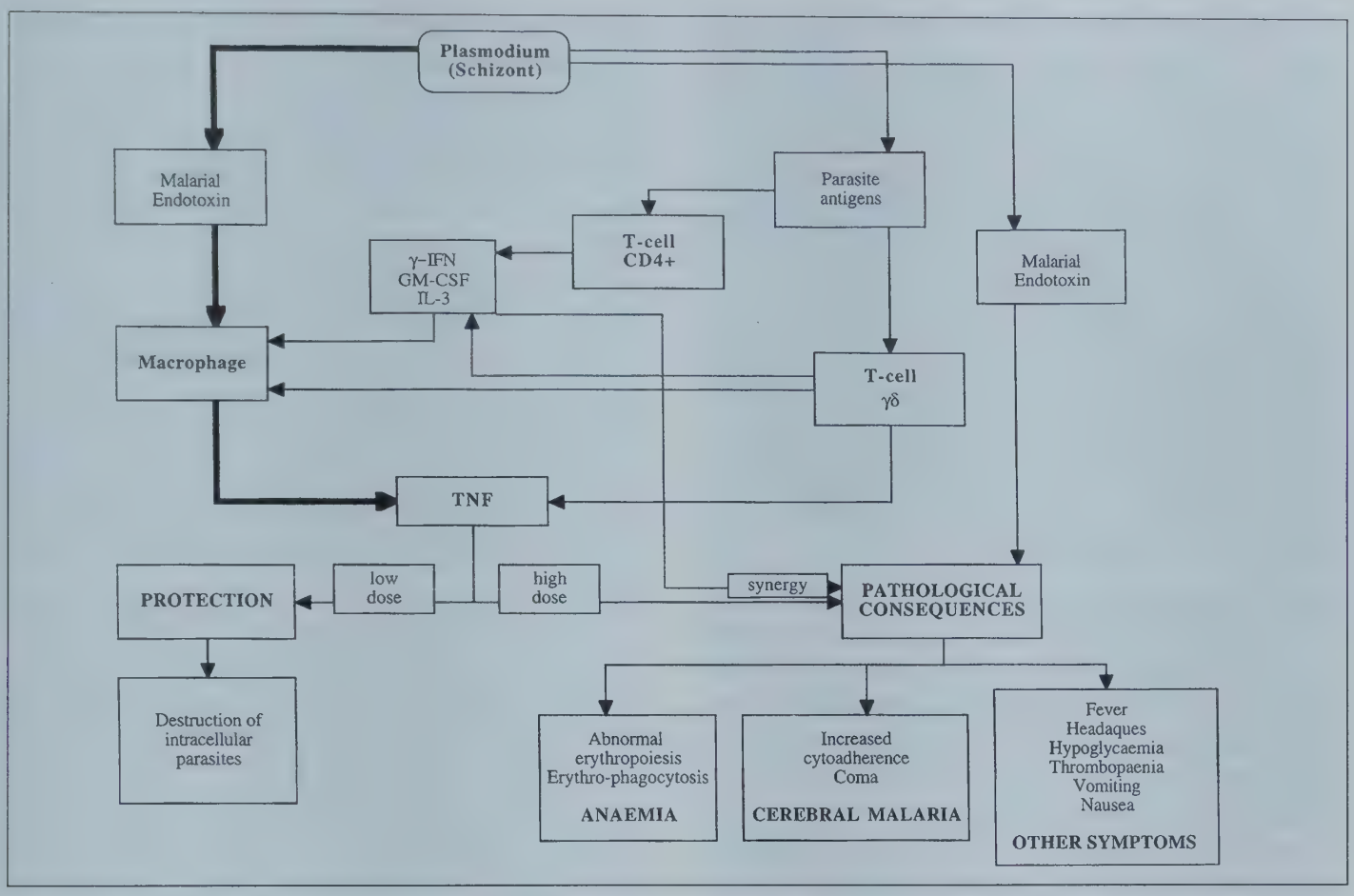

Fig. 2. Events leading to the induction of TNF-α during malaria and possible consequences of the presence of increased amounts of TNF-α for the patient.

may be interrupted in situations where malaria antigens stimulate predominantly TH2 $CD4^+$ cells, when the increased level of IL-4 interacts with IFN-γ production;

ii) the effects of some malarial antigens on γδ T cells, which respond by the production of high concentrations of IFN-γ and TNF. It is noteworthy that the massive increase in γδ T cells observed in naive individuals with acute malaria[20], does not seem to occur in individuals with acute malaria having lived continuously in endemic areas[21];

iii) the direct effect on macrophages of a special group of malarial antigens (the malarial toxins), which are probably lipids or proteolipids, structurally related to bacterial LPS, which are released either at the time of schizont rupture or immediately after (when the merozoite invades a red cell and sheds its surface coat). Candidate molecules include GPI anchor of a variety of malarial antigens (particularly MSP-1/MSP-2), malarial pigment or the residual vesicle containing pigment and a LPS-like antigen (Ag7). (For further information, the reader may refer to recent reviews by Jakobsen and colleagues and by Kwiatkowski)[22-23].

Effects of TNF

The following arguments have been put forward to support the hypothesis that TNFα plays a central role: the experimental injection of TNFα in humans produces symptoms that closely resemble a malarial paroxysm; the raise of temperature during *P. vivax* paroxysms closely follows the raise in circulating levels of TNFα; the levels of TNFα are higher in severe than in mild forms of Falciparum malaria and particularly high in fatal cases; the injection of anti-TNFα monoclonal antibodies to children with severe malaria abolish some of the symptoms, particularly fever; there are many similarities between severe malaria and sepsis, and TNFα and phospholipase-A2 are substantially increased in both.

At physiological levels, TNFα may destroy intracellular parasites or reduce the infectivity of gametocytes to mosquitoes; at increased levels, TNFα will induce fever, depress erythropoiesis and increase erythrophagocytosis thus contribute to anaemia and directly cause many of the non-specific symptoms of malaria. Although the circulating level of other "endogenous pyrogens", such as IL-1 and IL-6, is also increased during malaria[24], they appear to play only a modest pathophysiological role in severe malaria, probably in synergy with TNFα, as suggested by Rockett and colleagues[25].

Anti-disease immunity

Anti-disease immunity appears, therefore, to have two major components: the "*anti-toxic*" immunity itself and a necessary switch from a TH1 to a TH2 response. The former represents the specific response against malarial toxins which, because of the proteo-lipidic nature of the toxins is essentially a T-independent response; the ephemeral nature of such immunity would explain why a semi-immune adult, who leaves the endemic area for only a few months, is likely to develop an acute malarial paroxysm upon his return. Toxins of all malarial parasites are closely related and anti-toxic immunity is thought to be cross-reactive between species and strains of parasites[26]. The constant exposure to malarial parasites which appears to be required to maintain anti-toxic immunity may be achieved either by constant, long-term infection (as would be achieved by a parasite like *P. malariae*) or frequent re-infection (as would occur in a holoendemic area where the individual may be exposed to an excess of 300 infective bites per year). The idea that a long-term infection by *P. malariae* may be able to afford a degree of protection against pathology induced by a super infection by *P. falciparum* is recent and would explain certain features of anti-disease immunity in areas where mixed infections are common[27].

The switch from a TH1 to a TH2 response, which occurs at certain stages of infection, means that a different set of cytokines will be produced (including IL-4 and IL-10) which produces an environment less suitable for γδ T cells and macrophages to respond to malarial antigens by the production of TNF. Because a TH2 environment also interferes with the production of IFN-γ, this has the additional effect to reduce the possibility of a pathological synergy between IFN-γ and TNF. Evidence that a TH1 to TH2 switch occurs in man is best demonstrated by the studies of Mshana and colleagues in Gabon, where young children with acute malaria have high levels of IFN-γ, while the levels of IFN-γ decrease drastically in school-age children in whom IL-4 then becomes detectable[28].

It is likely that the asymptomatic state produced by anti-disease immunity in humans may be a prerequisite for the development of protective immunity: as long as infection is followed by an overproduction of inflammatory cytokines, the immune system is incapable of responding at the appropriate level to clear the infection; once this cascade is interrupted, protective anti-parasite immunity can rapidly develop. This would explain the paradox of a more rapid onset of anti malarial immunity in many experimental models, in which infection is frequently asymptomatic.

Premunition

The concept of anti-disease immunity, where the host requires to be more or less constantly exposed to malarial antigens, is reminiscent of that of "*premunition*", as was described by Sergent. However, the term premunition also refers to a situation where malarial infection is partially controlled by the host (i.e. a state of "relative immunity")[29]. In other words, premunition has elements of both anti-disease immunity (e.g. the existence of asymptomatic infections) and anti-parasite immunity (e.g. an age-dependent reduction of parasitaemia), hence the confusion the use of the term premunition has generated in the literature. It is true that the term describes quite well what happens to an individual after years of exposure to malaria (and, consequently, is helpful to epidemiologists), but modern immunologists have found it difficult to handle because of its ambiguous nature. Recent studies by

Baird in Indonesia have suggested that (in contrast to established dogma) a state of premunition may be more easily reached by adult migrants to an endemic area than by young children with lifelong residence in such an area[30].

Genetic differences in host susceptibility

Only a small percentage of infected individuals develop severe forms of malaria in endemic areas. This feature may be explained by an early acquisition of anti-disease immunity (while the infant is still protected by transferred maternal immunity) and/or by genetic differences in host susceptibility. Studies in the Gambia have recently shown that in a large cohort of children, those with the Class I HLA-B53 haplotype were less susceptible to severe malaria, while a relative resistance to severe anaemia has been associated with a Class II HLA-DR haplotype[31]; in the same cohort, an independent study has demonstrated that children with the TNF-2 allele of the TNF-α gene promoter were 7 times more likely to die of cerebral malaria or develop neurological sequelae[32]. While these results in a single Gambian cohort are sufficient indication that genetic differences in host susceptibility may exist, the role of the specific susceptibility factors described must not be over-emphasised since different very resistance/susceptibility characteristics may be found in other populations; indeed, the association of relative resistance with HLA-B53 was not found in a cohort study performed by the same research team in Kenya.

The sickle cell trait has been shown to be associated with a marked reduction of incidence of severe forms of malaria, which is such that malaria has actually been identified as a major selective pressure for the dissemination of the HbS trait[33].

Malaria in pregnancy

A paradoxical situation prevails during pregnancy in women with lifelong residence in a malarious area (e.g. having reached a state of semi-immunity): an apparent loss of immunity occurs during pregnancy, while at the same time, these women are able to transfer a fully effective protective immunity to their infant. This phenomenon is particularly pronounced in primigravidae and becomes less of a problem during later pregnancies.

The features of falciparum malaria in a primigravida are the following: infection takes place and the parasite develops in the placenta, while remaining at a low level in the peripheral circulation; in the absence of treatment, an infection acquired during the early stages of pregnancy will be maintained throughout pregnancy in the placenta, often with a heavy parasite load; the two major consequences of infection are a severe anaemia in the mother and a low birth weight in the infant; in extreme forms, there may be maternal death or abortion of the foetus. It is noteworthy that other forms of severe malaria (e.g. cerebral malaria) do not occur in this situation.

The classical explanation of what happens during malaria in first pregnancies is a reduction of acquired immunity, consistent with the immunosuppression generally observed in all pregnancies; this is in line with the observation of reduced lymphoproliferative responses in pregnant women compared to age-matched non-pregnant individuals[34], but crucial studies (e.g. the longitudinal follow-up of a cohort of women throughout their first pregnancy) have not yet been performed. The suggested loss of immunity does not account for the ability to transfer immunity to the infant (even if we accept that the described reduction of immune response is mostly in the cell-mediated response, while passive transfer refers mostly to antibodies).

Two alternative explanations for malaria in pregnancy have been proposed. The first takes account of the fact that the placenta is a "new" organ in primigravidae, which could by-pass existing immune responses in the host (without implying an actual loss of immunity) or allow for the development of unusual phenotypes of *P. falciparum;* the acquisition of a placenta-specific immunity or the previous exposure to new malaria phenotypes (in an earlier pregnancy), would explain the de-

crease susceptibility to infection in multigravidae. The second alternative suggests that what happens in pregnancy is a change in the balance of the local placental environment from an essential TH2 environment (prevailing in normal placenta) to a TH1 environment during acute malaria[35]; this is consistent with the presence of a large number of monocytes in infected placenta.

Immunopathology

The fact that the host's immune system appears to have considerable difficulties in controlling the malarial infection is probably the reason why so many aberrant responses have been described; aberrant responses are generally caused by an over-reaction of the host, where an immunologically inappropriate response has no effect on the parasite but may have immunopathological consequences of various degrees of severity. The major immunopathological syndromes encountered are severe malaria (including cerebral malaria and severe anaenia), hyperreactive malarial splenomegaly and quartan malarial nephropathy; in addition, malaria may contribute to the development towards Burkitt's lymphoma in areas of Epstein Barr virus (EBV) and malaria co-endemicity. Clinical and pathological descriptions of these various syndromes may be found elsewhere in this Handbook and a list of immunopathological complications of malaria is given on Table 2.

Cerebral malaria

Cerebral malaria (CM) is a severe complication of falciparum malaria and frequently leads to death, even when appropriate therapy has been given. Various hypotheses for the pathophysiology of CM

Pathology	Possible mechanisms
Hypergammaglobulinaemia	Antigen induced cytokine production (IL-6) Antigenic variation Polyclonal activation
Immunosuppression	Antigenic competition Structural disruption of germinal centres Disruption of spleen function Macrophage dysfunction Polyclonal activation and immune "exhaustion"
Nephrotic syndrome	Immune complex deposition Auto-immunity
Autoimmunity	Auto-antibodies Anti-nuclear antibodies
Anaemia	Anti-erythrocyte antibodies Dyserythropoiesis (e.g. effect of TNF) Excessive erythro-phagocytosis
Thrombocytopaenia	Excessive removal of platelets Coating of platelets with malaria antigen
Hyperreactive malarial splenomegaly	Genetic predisposition Hypergammaglobulinaemia Chronic increase of lymphocyte proliferation
Burkitt's Lymphoma	Co-endemicity with Epstein Barr Virus Polyclonal activation Antigen induced cytokine production

Table 2. Immunopathology of malaria. List of the various immunopathological complications of malaria and the mechanisms believed to be responsible for their pathogenesis.

have been proposed over time. While the theories implicating micro vascular obstruction by thrombus formation, disseminated coagulation, immunopathological events due to the deposition of immune complexes and local inflammation or the alteration of permeability with cerebral oedema have now been generally abandoned, the currently favoured hypothesis assumes a central role for intracapillary sequestration of infected erythrocytes (by cytoadherence to various endothelial receptors). The latter hypothesis has, at least in its earlier stages, tried to appertain the blame for CM entirely to the "stickiness" of infected erythrocytes, thus assuming that particularly *virulent strains* of *P. falciparum* were solely responsible for the evolution of infection towards CM. While there may be some elements of truth in this, it is becoming clear that it is the host contribution which plays the essential role; in particular, the role of TNF induction and the effects of TNF (perhaps in synergy with other inflammatory cytokines or with IFN-γ) in the upregulation of endothelial cell adherence receptors (e.g. ICAM-1) is now seen as the central feature of a cascade of events which will ultimately lead to CM (but only in certain susceptible individuals)[36].

Severe anaemia

The pathophysiology of anaemia is still poorly understood. It seems clear that in non-immune individuals anaemia cannot be satisfactorily explained by a destruction of erythrocytes as a result of parasite infection (since clinical disease occurs at a very low parasitaemia in such individuals). This is different in individuals having lived from some years in malarious areas, who have acquired a degree of anti-disease immunity, hence a "*tolerance*" to high or very high parasitaemia; such individuals will be suffering from a form of anaemia directly related to the degree of erythrocyte destruction, but such anaemia in older children or adults is not considered a serious health problem. The main problem in malaria is the severe anaemia which occurs in early childhood before immunity is effective. This form of severe malaria occurs at low parasitaemia and is accompanied by substantial dyserythropoiesis. TNF is presumed to represent a major aggravating factor in anaemia, by stimulating erythrophagocytosis and depressing erythropoiesis in the bone marrow. Of interest is the reported difference in age-prevalence of severe anaemia (in early childhood) and cerebral malaria (in children over 2 year old)[37], which is consistent with the view that the pathogenesis of the latter requires a greater immunological component (e.g. synergy between TNF and IFN-γ) than the former.

Hyperreactive malarial splenomegaly

In malarious areas, children exposed to repeated infection develop a marked splenomegaly; as their immunity to malaria develops, there is a progressive reduction of the size of their splenomegaly. The determination of a splenic index is therefore not only a useful epidemiological tool, but also gives a rough estimate of the degree of age-related immunity in a population. However, some adults who have acquired an apparently normal state of semi-immunity continue to have a marked splenomegaly; this was long known as the "*tropical splenomegaly syndrome*", but is now usually referred to as "hyperreactive malarial splenomegaly" (HMS). The precise pathogenesis of HMS has not yet been elucidated, but it has been suggested that this is a truly immunopathological phenomenon, consisting of an aberrant humoral response to malarial antigens (probably due to a clonal proliferation of some sets of B cells) and occurring only in genetically susceptible individuals. Apart from the fact that elevated IgM is a constant feature, the precise nature of the immunological defect and the genes controlling susceptibility have not been clearly established (see review by Marsh and Greenwood[38]).

Quartan malarial nephropathy

The frequency of nephrotic syndrome appears to be 100 times higher in African than European or American children. Renal immunopathology has been demonstrated in many animal models with malaria as well as in acute *P. falciparum* malaria

in man. However, it is the nephropatic syndrome associated with *P. malariae* which is usually reported in this context[39]. The syndrome appears to be caused by the deposition of *P. malariae*-antibody immune complexes, but the reason why such complexes are formed in some individuals and why they are deposited remain mysterious. The reader should be reminded that this fascinating association has now been described over 20 years ago, at a time when immunology was still in its infancy and that it has not been given much attention since (despite the fact that *P. malariae* is as common in West Africa now then it was then). The major difference between the nephropathy observed in acute falciparum malaria and that in chronic malariae malaria is that the former is essentially reversible with antimalarial treatment, while the latter is resistant to treatment and has often a fatal outcome.

Burkitt's lymphoma

The effects of malaria on host immunity, which include a mixture of intense proliferation of lymphocytes directed against malarial antigens and an immunosuppression of mechanisms controlling polyclonal proliferation, creates an environment which is more suitable for the proliferation of some viruses. This has been shown to be true for EBV and, in areas of co-endemicity, malaria is believed to be a major provoking factor in the evolution of EBV infection towards the malignant Burkitt's lymphoma[38]. It is perhaps surprising that a similar situation does not exist with regard to HIV-1 infection, since no study has so far managed to demonstrate an acceleration of the progression of infection towards AIDS or an increased severity of malaria in AIDS patients.

In vitro correlates for immunity

In view of the long period of time which is required for an individual to acquire a state of effective protection against malaria, it would be helpful to possess markers to measure the stage of immunity reached by an individual at a given time (e.g. is it anti-disease immunity alone, anti disease plus a relative anti-parasite immunity, or strain-transcending anti-parasite immunity?); at present none of these stages has been correlated with any measurable clinical or laboratory parameters and it is not even certain that this sequence of events always follows the same pattern. The need for measuring protective immunity is also relevant in the context of vaccine development, since the existence of reliable criteria for protection would enable the scientists to draw conclusions regarding the potential value of a vaccine candidate without having to perform cumbersome challenge experiments in human volunteers.

Ideally, the parameters to be measured should be *in vitro* assays measuring a particular effect of immunity on the parasite life cycle. *In vitro* assays could measure one of the following biological function: i) interruption of sporozoite invasion (incl. circumsporozoite precipitation test, inhibition of hepatocyte invasion in culture, surface immunofluorescence of fixed ring-infected erythrocytes); ii) interruption of merozoite maturation and invasion (incl. inhibition of *in vitro* culture, inhibition of merozoite dispersal, merozoite agglutination, inhibition of erythrocyte invasion in culture); iii) inhibition of parasite growth in erythrocytes; iv) immune response to erythrocyte-associated malarial antigens (incl. surface immunofluorescence of live schizont-infected erythrocytes, agglutination of schizont-infected cells, inhibition/reversal of cytoadherence; inhibition/reversal of rosetting); or iv) effects on gamete maturation or fecundation (incl. gamete agglutination, inhibition of gamete fecundation or inhibition of öocyst production in mosquitoes); a majority of these assays and their value have been discussed by Kreier and Green[40]. Alternatively, *in vitro* correlates could concentrate on the antigenic aspect of parasite molecules (or their recombinant or synthetic equivalent); such assays may measure only the degree of immune recognition of the molecule (e.g. by ELISA using a synthetic peptide such as $NANP_3$ for measuring a response to CSP, $EENV_6$ for measuring a response to RESA or $SVTEEIAEEDK_2$ for measuring a response to Ag332) or measure an effective immunoproliferative response of patient lymphocytes to such an antigen[17].

A number of assays have shown a pattern of response which is suggestive evidence that the assays may measure protective immunity (particularly a response increasing with age and the degree of exposure to malaria): this pattern has been observed for a number of epitopes of a number of malarial antigens (particularly CSP, RESA, GLURP, MSP-1). In one of the rare studies where a number of such assays have been compared to one another, in a vertical study of a cohort of Gambian children of different ages, the results were disappointing and none of the assays proved to provide a better prediction of the state of protective immunity than IFAT (using whole malaria parasites and measuring the overall response to the parasite)[41].

In contrast to *in vitro* assays which aim to provide a correlate for protective immunity, most of the immunoassays used for immunodiagnosis measure a response to malarial antigens which may not be relevant to protective immunity (i.e. irrelevant immune response to abundant and immunogenic antigen which may be inaccessible to effector mechanisms of immunity).

Immunodiagnosis

Malaria is a disease without characteristic symptoms and diagnosis generally relies on finding the parasite, based on clinical suspicion or evidence of recent exposure to infection. A reasonable set of guidelines for diagnosis would include: a clinical illness compatible with malaria, including objective evidence of fever, the exclusion of any other likely cause of fever following physical examination and a parasitaemia above 10,000/µl. It is probably still true to say that, despite considerable progress in immunology and molecular biology, microscopy remains the only certain means of diagnosing all four species of human malaria. The main problem with microscopical diagnosis is that it is a time-consuming technique which needs to be performed by skilled microscopists. Instead of identifying the parasite itself, immunological methods provide the means for detecting either the parasite antigens or host antibodies directed against it. While the detection of antigens may be an acceptable alternative to parasite detection, particularly if the assay is robust, cheap, easy to use in field conditions and does not require a microscope, the detection of antibodies merely provides information on the past malaria experience of an individual and is of limited use for individual diagnosis.

Serology

Serological methods have been in use since the early 1960's, when indirect fluorescent antibody test (IFAT) and indirect haemagglutination assays (IHA) were described. IFAT is the main method for routine serodiagnosis, because it is relatively easy to make antigen slides for all human malarial parasites (commercial IFAT slides for malaria are also available from various sources); its disadvantages are the requirement for a fluorescence microscope, the subjectivity of the reading and the fact that the method is relatively labour-intensive, which limits its application to specialised centres with a relatively small through-put of samples. ELISA uses a soluble malarial antigen (generally prepared from asexual stages of *P. falciparum*, which can be readily grown *in vitro*), coated on the wells of a microtitre plate; this enables a large number of samples to be processed at the same time and produces quantitative results. In addition to crude extracts of malarial antigens, ELISA has been applied to a variety of defined, synthetic or recombinant malarial antigens (e.g. MSP-1, RESA or CSP); such studies have been useful for elucidating the role of target malarial antigen in immunity and protection. A variety of other serological test formats have been explored, including radioimmunoassays, latex agglutination, indirect haemagglutination, solid-phase dipstick and membrane dot-blot, which may have specific advantages in given situations. The reader wishing to know more about malaria serology may consult the extensive review of the subject by Gilles[42].

Antigen detection

In contrast to serology, a positive antigen detection assay would be expected to coincide with a current infection. The best antigen detection assays described have

a maximum sensitivity of 0.01 to 0.001% parasitaemia and are, therefore, of a sensitivity 5-10 times inferior to that of a good quality microscopy. ParaSight™-F[43] is a commercialised detection test in dipstick format, in which a monoclonal antibody captures a specific antigen of *P. falciparum* (HRP2); if antigen is present, the positivity of the test is visualised by a second anti-HRP2 antibody labelled with a coloured marker, which produces a visible line on the dipstick. The whole test only takes 10 minutes to perform and gives a sensitivity almost comparable to that of thick films. This simple, robust assay requires no equipment and can be taught to village health workers, since its reading is a straight-forward positive/negative assessment. Its high cost and the fact that the assay is not quantitative in its current format, are serious limitations to its use in the very field situations for which it was designed.

While of limited use for individual diagnosis, immunodiagnostic methods may have a place in two situation: epidemiology and the screening of blood for transfusion. Active or passive surveillance of malaria prevalence is an important tool for malaria control, particularly when the efficacy of control measures are being evaluated. Microscopy and, by extension antigen detection (or PCR), have serious limitations for such an evaluation, because these techniques only measure a "point prevalence" of the infection at the time of the survey. In contrast, *sero-epidemiology* may help to delineate those areas where there is malaria transmission, provide information on species-prevalence or age-related prevalence, and chart the changes that are taking place as a result of a control intervention. The transmission of malaria by *blood transfusion* is a serious risk, since the diagnostic of malaria in the recipient, being unexpected, is often missed. Microscopic examination of donor blood is highly unsatisfactory since most donor infections are at a sub-microscopic level. Outside endemic areas, the policy for screening the donor's history for known episodes of clinical malaria or for tropical travels in the past five years is generally sufficient, but in view of the increasing frequency of tropical travels, the policy in some countries is to reject only donors whose malaria serology is positive (generally using IFAT with homologous antigens for *P. falciparum*, *P. vivax* and *P. malariae* for maximum assay sensitivity). This screening method is not perfect, but a negative serology gives a high probability of freedom from infection. In endemic areas, the only safe prevention of transfusion malaria is appropriate preventive antimalarial therapy of the recipient.

Other issues relating to malaria diagnosis have been dealt with in Chapter "Diagnosis of malaria infection" of this Handbook.

Evasion from host immunity

Protective immunity against malaria parasites is slow to develop and a child born in a malaria endemic zone may need to be exposed to successive infections for years before developing resistance to the parasite; complete protection may never be achieved. This "real-life" situation is in sharp contrast to what is observed in experimental situations where a solid, sterile immunity can be achieved against the parasite population used. The apparent inefficiency of antimalarial immunity may be explained by the ability of the malaria parasite to evade host immunity either by the intrinsic poor immunogenicity of its antigens, by sequestration, by antigenic diversity and variation, or by an alteration of the host's immune response.

Poor immunogenicity

Crucial antigens involved in protection may be poorly immunogenic either because of their intrinsic molecular structure or their analogy to host molecules, or because of immune restriction which may impair recognition. The presence of multiple repeat sequences on a number of highly immunogenic antigens (e.g. RESA, S-antigens or FIRA) has been interpreted as an evasion mechanism, since such immunodominant structures may act as a "smokescreen" preventing the development of effective immunity to more relevant epitopes. The existence of cross-reactivity between repetitive epitopes of different malarial antigens (e.g. epitope

shared between RESA, FIRA, Pf11.1, Ag332 and an S-antigen) is a further cause for poor immunogenicity since the presence of these epitopes may interfere with the normal maturation of the immune response towards progressively increasing higher affinity antibodies. While it is probable that different individuals may be more or less responsive to malarial antigens (e.g. because of MHC Class II restriction), this factor is probably less important with whole molecules than for short peptide epitopes (e.g. in the context of peptide vaccines).

Sequestration

The ability of parasites to remain sequestered by cytoadherence to the capillary lining of certain tissues must be regarded as a selective advantage since such parasites can avoid frequent passage through the spleen and thus exposure to immune effector mechanisms. *P. falciparum* isolates are able to switch rapidly from one endothelial receptor to another (e.g. switch serially from CD36 to ICAM-1, ELAM-1 and VCAM-1) and this may be part of a parasite survival strategy, particularly since the change of receptor requirements may be in response to a changing environment of the host.

Alteration of the immune response

There are several reports which demonstrate that malaria infection can induce a suppression of the host immune responsiveness (including the increased severity of salmonella infections, the decreased efficiency of tetanus vaccination and the association between malaria and Burkitt lymphoma). Various mechanisms of immunosuppression have been proposed, including polyclonal activation, macrophage dysfunction, abnormal antigenic presentation, disruption of lymphatic and splenic tissue architecture, activation of suppressor cells and antigenic competition.

Antigenic diversity and antigenic variation

The considerable antigenic polymorphism of malarial parasites and their ability to change crucial surface antigens, either spontaneously (as is the case for PfEMP-1) or in response to a strong immune pressure, provides the parasite with a powerful means for escaping from host immune responses (see above).

Malaria vaccines

The prevention of malaria by vaccination is a conceivable approach, which may eventually have a place in malaria control. Unfortunately, no effective malaria vaccine is as yet available and, despite very intensive research since the mid-70's, none is likely to be available at an operational level for many years. The topic of malaria vaccines is vast and complex and has been reviewed at length elsewhere[44, 45]. The aim of a vaccine is to reduce morbidity/mortality due to malaria and this may be achieved in one of two different ways: either by interrupting the infection at one or other of the stages of the parasite life-cycle, or by means of an *anti-disease* vaccine, which would reduce the pathophysiological effects triggered by the release of malarial toxins (as proposed by Playfair and colleagues)[46]. The interruption of the life-cycle may take place at the time of entry of the sporozoite into the host, at the level of schizogony in the hepatocyte, at the time of erythrocyte invasion and intra-erythrocytic parasite development, or by interruption of the sexual development within the mosquito.

The methods by which host immunity will control the infection are variable, depending on the target and this will have an implication on the choice of immunogen. For example, a pre-erythrocytic vaccine is likely to rely more on the production of CTLs than antibodies, while a transmission-blocking vaccine is likely to rely essentially on antibodies to sexual stages; it follows that new methods for immunisation using "naked DNA" are likely to be more useful for pre-erythrocytic stages (since they are effective in producing CTLs) than for transmission-blocking vaccines.

A number of vaccine candidates has been tested in human volunteers with variable success: the best results were obtained with X-irradiated sporozoites, while synthetic and recombinant antigens have

so far given disappointing results. The Spf66 vaccine is the only one so far to have reached Phase III clinical trials, but it gave disappointing results: a 31% efficacy was reported from the first trial in Tanzania[47], but only a 3% efficacy, when tested in The Gambia[48]. Current research trends in vaccine development are geared towards "cocktail" vaccines including a combination of multiple epitopes from different malarial antigens and from different stages of the parasite life-cycle. Any form of crystal-gazing today suggest that all future trials are likely to be multi-centre trials of a multi-stage, multi-epitope vaccine; the antigens most likely to be considered for such trials will include a combination of CSP, LSA-1/3, MSP-1/2, SERA, AMA-1, Pfs48/45, Pfs25, STARP, SSP-2.

The predicted impact of a malaria vaccine in controlling the disease has been discussed and analysed with a variety of mathematical models and with various degrees of optimism. Even if a perfect vaccine can be found, it is unlikely to solve malaria on its own, but will need to be intelligently integrated into an appropriate control strategy, adapted to each endemic situation.

Immunity and chemotherapy

A number of issues have to be considered:

i) does chemoprophylaxis interfere with the development of immunity?
ii) are drugs more effective in semi-immunes than in naive individuals?
iii) does drug pressure contribute to the antigenic diversity of malaria parasites?
iv) do antimalarial drugs have an effect on the immune status of the patient?

It seems well-established that appropriate chemoprophylaxis, taken regularly (such as is done by expatriates residing in an endemic area) interferes with the development of immunity. The situation is more confused when the effects of targeted mass chemoprophylaxis are studied (e.g. in the Garki Project in Nigeria or, more recently, in The Gambia): young children receiving chemoprophylaxis are not fully protected from malaria infections, but infections are less heavy, less frequent and there is a substantially reduced mortality and morbidity[3, 49]. In these children, antibody levels are generally significantly reduced, but reduction is less marked the longer the chemoprophylaxis is given; in contrast, lymphocyte proliferation or γ-IFN responses are increased suggesting that reduced infection levels generate more effective cell-mediated responses. Once chemoprophylaxis is interrupted, there does not appear to be a "rebound" effect in clinical attacks, morbidity or mortality, which suggests that children having received some form of chemoprophylaxis over the first few years of life (and thus suffered less from their malaria) have nevertheless developed an anti malarial immunity comparable to that of children without chemoprophylaxis. In any situation where mass chemoprophylaxis is associated with other effective means of prophylaxis (e.g. impregnated bednets), the chance of infection may be so substantially reduced that the development of immunity is impaired[50].

Anti malarial drugs may act much more effectively in semi-immune than in naive individuals, due to a synergy between the effect of drugs and immunity. A consequence of this is that, in geographical areas where there is resistance to a given drug, the clinical response may be very different in different age groups (for example, in a holoendemic area, there may appear to be a lower degree of drug resistance in school age children than in infants). Another consequence of this is that, in hyper- or holoendemic areas (where most of the population reaches a state of semi-immunity from a relatively early age), drugs like chloroquine may continue to be of benefit to the local population even when a relatively high degree of parasite resistance has been reported. This has implications in the interpretation of *in vivo* drug susceptibility tests and explains some of the discrepancies observed between *in vitro* and *in vivo* tests[51].

The hypothesis that drug pressure may act as a selective pressure to facilitate parasite antigenic diversity and suggesting that drug resistant parasites may be antigenically different from sensitive ones[52-53] is

a controversial one. While it is likely that continuous drug pressure in a population may contribute to the emergence of parasites with a drug resistant phenotype, there is now convincing evidence to indicate that the parasite has sufficient intrinsic ability for generating antigenic diversity on its own and that the immune pressure alone provides a sufficient selective pressure. There is no experimental evidence to support the view of significant antigenic differences existing between sensitive and resistant strains of the parasite. Much has been written regarding both the immunosuppressive and the immunostimulant activity of antimalarial drugs, which is outside the scope of this paper (see review by Peters[52]).

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PATHOLOGY OF MALARIA

H. M. Gilles

Introduction

The pathological changes in malaria are related to the development of asexual parasites in the blood. In *P. falciparum* infections, the multifaceted nature of the interaction between the erythrocyte, the host immune system and the parasite is central to the pathogenesis of severe malaria and results in mechanical and rheological changes to the infected erythrocyte. These modifications lead to knob protrusions, cytoadherence and rosette formation. Parasitised erythrocytes bind to host molecules CD36, ICAM-1, thrombospondin, E-selectin, VCAM-1, chondoctrin sulphate (Hommel, 1993)[1].

The release of malaria antigens, pigment and toxins gives rise to a cascade of pathological events. Among these the production of cytokines, particularly tumour necrosis factor (TN Fα), induced by the release of parasite products during schizont rupture, appears to play a central role; complemented by the effects of other circulating "endogenous pyrogens" such as interlukin-1 (IL-1) and IL-6. TNF or cachexin has been implicated as the cause of malarial fever. Although the nature of malarial toxin is still controversial, it is generally agreed that it is released at the time of schizont rupture. Excellent reviews on the pathogenesis of malaria have been published (Miller *et al.,* 1994; Pasvol *et al.,* 1995)[2, 3].The immunopathology of malaria is further considered in another chapter.

Pathology of the individual organs

In spite of the many forms of malignant tertian malaria that are known, death from *P. falciparum* in children living in areas of stable malaria is usually due either to cerebral malaria, malarial anaemia, metabolic acidosis, or a combination of these; whereas death in non-immunes are often associated with acute renal insufficiency, cerebral malaria, pulmonary oedema and disseminated intravascular coagulation. In any description of the pathological changes of malaria, two situations must be borne in mind namely (1) acute lesions responsible for death in the non-immunes (2) changes occurring in the organs of the partially immune child or adult who has died from other causes.

Parasitisation is greatest in descending order in the following organs: brain, heart, liver, lung, kidney and blood (Edington, 1967; Edington & Gilles, 1969; Francis & Warrell, 1993)[4, 5, 6].

Central nervous system

Although changes have been reported in the spinal cord and peripheral nerves, the most marked changes are seen in the brain itself. The major feature of cerebral malaria is the existence of cytoadherence of parasitized erythrocytes to the endothelium of cerebral capillaries and venules, resulting in the sequestration and tight packing of infected cells in these vessels.

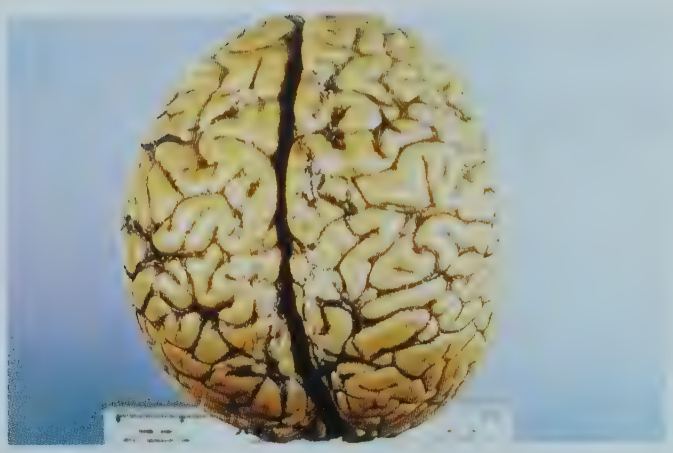

Fig. 1. Leaden appearance of brain in cerebral malaria.

Fig. 2. Cerebral malaria. Numerous petechial-haemorrhages are shown scattered throughout the white matter.

Fig. 3. Ring haemorrhage in cerebral malaria.

The meninges are grossly congested, the smaller vessels being packed with parasitized cells. A perivascular lymphocytic infiltration has been described. The brain itself may show gross congestion only, but it is usually leaden in colour (figure 1) – the smaller vessels of the grey matter being packed with red cells containing pigmented parasites in all stages of development. Gross congestion of the vessels is invariable, and in the majority of instances numerous petechial haemorrhages are evident in the white matter of the cerebrum, brain stem and cerebellum (figure 2).

Haemorrhages are not usually seen in the grey matter although they can occur there; this lesser liability to haemorrhage is thought to be due to the greater number of capillaries and anastomatic channels in this area of the brain as compared with the white matter. Histologically the capillaries and arterioles are packed with parasitized cells, and ring haemorrhages are a striking feature. These consist of a central 'blocked' vessel (most frequently an arteriole), containing an agglutinated mass of parasitized erythrocytes surrounded by brain tissue, and then by a ring of extravasated red blood cells (figure 3). Parasites are sometimes present at the periphery of these haemorrhages.

In older haemorrhages necrosis of the midzonal brain tissue occurs, and there is a peripheral reaction of small glial cells - the so-called malarial granuloma. Healing is said to cause subsequent scarring possibly with residual brain damage.

Death can occur in cerebral malaria with few parasites in the majority of the cerebral vessels. In these cases parasitized erythrocytes have been seen in the central vessels or in the erythrocytes in the ring haemorrhage, and a history of treatment prior to death has usually been obtained.

In deaths due to malarial anaemia there is pallor of the brain and its coverings, the vessels are empty and pigment has not been seen in the endolethial cells of the vessels.

Anaemia

This is an inevitable consequence of erythrocyte parasitization but the mechanism is multifactorial and complex. The following points are established (1) para-

sitized and unparasitized cells are phagocytosed and destroyed; (2) anaemia is not necessarily related to the degree of parasitaemia; (3) transfused cells in a malarial patient may be destroyed more rapidly than in a normal recipient; (4) iron-sequestration and dyserythropoiesis occur; (5) erythropoiesis in the bone marrow is depressed. TNF stimulates erythrophagocytosis and bone marrow depression of erythropoiesis and is considered an important aggravating factor in the pathogenesis of anaemia.

In the acute stage there may be few changes in the morphology of the red cell other than those changes initiated by the parasite itself. In other instances, however, anaemia may be marked and the findings in the peripheral blood thus depend on the duration and stage of the infection. It must be remembered that malaria is a potent cause of severe anaemia in children from the age of 6 months to at least 2 years in areas of stable malaria and it can be sufficiently severe as to cause death.

The anaemia is haemolytic and in the acute attack there may be a sudden and dramatic fall in the haemoglobin values of the blood. It is usually normocytic and normochromic, or hypochromic but macrocytic if there is a marked reticulocytosis or if folic-acid deficiency eventuates - a not uncommon complication in tropical areas. The causes of folic acid deficiency in such patients are multiple: (1) inadequate dietary folate; (2) reduced absorption of folic acid; (3) increased utilisation due to haemolysis and fever of malaria. The peripheral blood film shows many parasites, polychromasia, anisocytosis, poikilocytosis, target cells, basophilic stippling and, in severe cases, Cabot's rings, Howel Jolly bodies, and nucleated red cells. A reticulocytosis may be present but this is more usual as a result of treatment.

Thrombocytopaenia is common in both *falciparum* and *vivax* malaria; platelet survival is reduced; enhanced splenic uptake and sequestration occurs.

Mild leucopenia is usual in uncomplicated malarias but leucocytosis is an important abnormality in severe malaria and is associated with a bad prognosis.

Haemozoin (malarial pigment) is commonly present in the monocytes and may occur in the polymorphonuclear leukocytes as well. Haemosiderin, a dark yellow trivalent iron-containing pigment, is formed in the reticulo-endothelial system from the breakdown of haemoglobin contained in red cell debris liberated during schizogony, from senescent and parasitized cells, and from unparasitized cells haemolysed or phagocytosed during the acute attack. It is deposited mainly in the spleen, liver and marrow. This pigment can be differentiated from haemozoin in tissue in that it gives a positive Prussian blue reaction. The haemosiderin formed by the reticulo-endothelial system from the breakdown of haemoglobin is immediately available and is re-utilised in the synthesis of the large amounts of haemoglobin necessitated by the haemolytic process.

Bone marrow

The bone marrow is greyish red, soft and hyperaemic and is increased in the long bones. In the acute stages its vessels are full of parasitized erythrocytes and haemozoin is present in the reticulo-endothelial cells and monocytes. There is a marked normoblastic hyperplasia even in the absence of a reticulocytosis in the peripheral blood and there is also myelocytic proliferation. Some authors, however, have demonstrated a temporary inhibition of the marrow during parasitaemia. Megaloblastic change may result if folic-acid deficiency occurs. Increased numbers of large abnormal-looking megakaryocytes are found in the marrow.

The spleen

In the acute attack the spleen is enlarged, and tense, and the cut surface is slaty greyish red with the malpighian corpuscles prominent (figure 4). The consistency may be soft if a terminal bronchopneumonia is present. Histologically the blood vessels, Billroth cords, and sinusoids are filled with parasitized red cells. Parasitized and unparasitized cells and haemozoin are seen in the pulp histiocytes and sinusoidal lining cells. Pigment may be found lying free in the pulp and sinusoids, and it can also be found in the

Fig. 4. Spleen showing slaty greyish-red appearance.

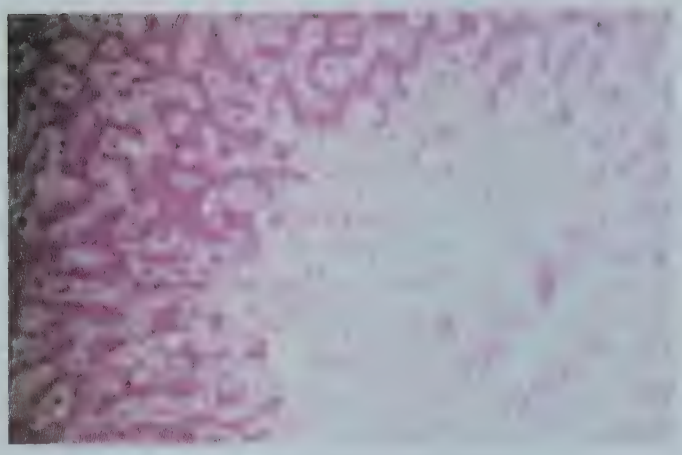

Fig. 5. Liver – Centrilobular necrosis.

germinal follicles. A splenic smear reveals developing forms of parasites and haemozoin lying free and contained in monocytes. Degeneration of the endothelial cells of splenic vessels may occur causing thrombosis, haemorrhage and infarction.

With increasing immunity the spleen becomes at first jet black with much pigment in the cords, but gradually the congestion decreases and the pigment disappears first from the sinusoids and last from the cords with parasitized cells becoming scanty. The spleen diminishes in size, the capsule becomes greyish, fibrotic, and wrinkled, perhaps with some evidence of old-standing perisplentitis, and some fibrosis is seen in the pulp.

Rupture of the spleen is a not uncommon complication of malaria and usually occurs through the hilar region. It should be emphasised that, if such an accident occurs and splenectomy is performed for this or any other reason in an immune individual living in an area of stable malaria, continuous suppressive antimalarial therapy must be considered to prevent the possible development of a severe malarial infection.

The liver

The pathological changes in the liver vary according to the immunological status of the individual and the mode of death. In cerebral malaria the liver is enlarged and tense and its colour varies from dark red to slaty grey. If, however, anaemia has been gross the liver is enlarged and pale yellowish grey in colour.

Histologically the striking feature in the acute stage is the gross congestion of the sinusoids and centrilobular veins by parasitized erythrocytes. The Kupffer cells are hypertrophied and contain parasitized and unparasitized red blood cells, remnants of parasites and granules and masses of haemozoin, with haemosiderin inconstantly present. The parenchymal cells may contain haemosiderin but never haemozoin. One of the most striking and constantly reported features has been degeneration and necrosis in the centrilobular regions in the absence of heart failure (figure 5). This feature was not noticeable in autopsies on children dying of cerebral malaria in West Africa (Edington, 1967)[4].

The kidneys

In falciparum malaria an acute and transient self-limiting glomerulonephritis is common, whereas in *P. malariae* a chronic glomerulonephritis presents as nephrotic syndrome. In blackwater fever, large amounts of haemoglobin are cleared by the kidney following intravascular haemolysis. This may lead to oliguric or anuric renal failure. In severe malaria, there is gross congestion of the vessels (figure 6) with parasitized erythrocytes, especially in the capillaries of the glomerular tuft. The histological changes are those of acute tubular necrosis due to reduced cortical perfusion.

Pigment has been described in vessels, free in the interstitial tissue, occasionally in the epithelial cells of the tubules, and within phagocytes in the capsular spaces. It

Fig. 6. Kidney – Medullary congestion in severe malaria, resulting in acute tubular necrosis.

has also been described in both the epithelial and endothelial cell of the glomeruli. Hyaline, epithelial, and granular casts may be present in the tubules. Scattered small haemorrhages may be seen in the cortex and medulla.

An association between the nephrotic syndrome in children and *P. malariae* infection has been established (Gilles & Hendrickse, 1963)[7].

P. malariae results in a nephropathy of immune complex origin with microscopical patterns ranging from minimal change to membranous, the latter referred to as "quartan malarial nephropathy" (Hendrickse *et al.*, 1972)[8].

Adrenals

Changes in the adrenals are variable. Degenerative and necrotic changes in the inner zone of the cortex with loss of lipid have been described. The more usual finding, however, is gross congestion and haemorrhage.

The lungs

The smaller vessels are packed with parasitized erythrocytes and small haemorrhages may be present. Hyaline membrane formation, thickened alveolar septa and areas of alveolar haemorrhage have been described. The alveoli are congested with pigment-laden macrophages, plasma cells, neutrophils and parasitized erythrocytes.

In Spitz's autopsy series, pulmonary oedema was a universal finding; when not iatrogenically produced, it often resembles the picture seen in the adult respiratory distress syndrome (ARDS). The basic lesion appears to be injury to the capillaries of the lung with congestion and leakage of oedema fluid.

The cardiovascular system

The usual picture is one of vessels congested with parasitised erythrocytes, pigment-laden macrophages, lymphocytes and plasma cells. There is little evidence of cytoadherence. Small subendocardial haemorrhages may occur.

Fatty degeneration of the myofibrils and brown atrophy have been described. In severe malarial anaemia in children the effects are those resulting from increase in blood volume and anoxia.

Gastro-intestinal tract

Congestion with capillary stasis, necrosis, mucosal ulceration and haemorrhages can occur. Sequestration and cytoadherence have been seen, both in the small and large bowel, especially in the lamina propria capillaries. Malabsorption of amino-acids, sugars and fats have been described. Absorption of antimalarial drugs is generally adequate.

Placenta

It is black or slaty grey and the sinusoids are packed with infected erythrocytes (figure 7).

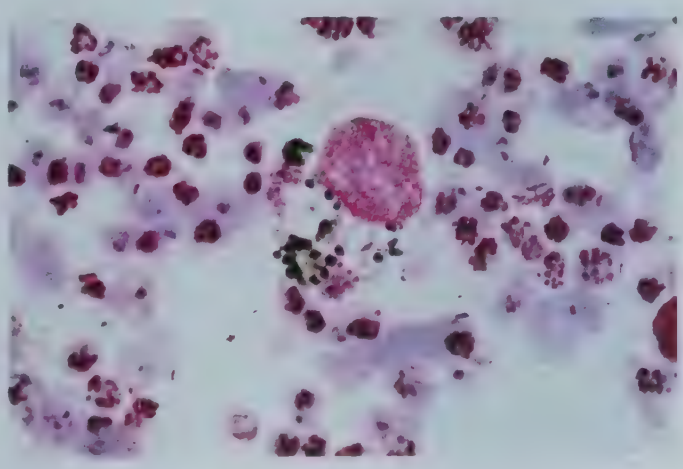

Fig. 7. Placenta – Massive placental parasitisation in a primigravidae.

Developing trophozoites are numerous in the intervillous spaces and are found in the greatest numbers next to the trophoblast of the stratum spongiosium, and haemozoin may be seen within the fibrin masses, in some instances surrounding degenerate villi. It would appear that the 'stickier' parasitized cell tends to 'sludge' in the eddies of the slow-moving placental stream. Pigment is seen in the fibrin. This most probably favours fibrin deposition on the villi thus hastening the degenerative processes, interfering with the nutriment of the fetus and causing stillbirths and premature labour. There is an increase of cells – mainly histiocytes – in the maternal sinuses of the placenta. The maternal blood in the intervillous spaces is high in glucose content – favouring the development of the parasite (WHO, 1996)[9]. The mechanism by which placental parasitisation affects foetal growth is not known.

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CLINICAL ASPECTS OF MALARIA

J. Van den Ende, A. Van Gompel

Introduction

Malaria has a variety of clinical pictures, from acute to chronic, and from simple fever to life-threatening multiple organ failure. The clinical picture differs with the species of parasite involved, but also with the immune status of the patient. *P. falciparum* is by far the most dangerous, with the most dramatic symptoms and signs.

Clinical syndromes like black water fever, cerebral malaria, hyperreactive malarial splenomegaly are recognised as distinct entities. In recent years minor, atypical malaria syndromes have been recognised. The impact of these syndromes on the general health in endemic countries is difficult to measure, but could be substantial. Therefore, studying malaria in expatriates returning from endemic countries is important in establishing the relationship between infection and clinical patterns. Translating these findings to malaria in endemic regions however should be done with caution.

Every patient can tell you "his" symptoms of malaria, and the proof of infection is given by the rapid decline in symptoms while taking treatment. This argument is weak: firstly because a lot of viral diseases heal spontaneously and secondly because some antimalarials are also antiphlogistics, although with delayed action.

There is no definitive, unique indicator of morbidity, hence no diagnosis is as controversial as malaria. The boundary between malaria "infection" and malaria "disease" is not clear. The finding of parasites in a blood smear does not necessarily mean that the patients' symptoms are due to malaria, as the presence of parasites is required to build up and to maintain an immunity, which is never complete. Quite accurate techniques have been developed to determine the impact of malaria within populations, and the level of parasitemia that is required to distinguish symptoms due to other diseases from symptoms due to malaria infection (see chapter "Diagnosis of malaria disease"). However, is the diarrhoea of an individual patient provoked by malaria infection, when one finds parasites in the blood, or is a different pathogen responsible? And what if no pathogen is found? This is an endless topic of debate and research, as scientists try to establish a pattern of symptoms and signs, or a marker that could unequivocally point to malaria as the cause of the patient's complaints.

To our knowledge, no other disease requires such a long time and demands such a high price for an incomplete and transient immunity. In most endemic countries, children will suffer recurrent attacks from a few months after birth until the age of 5 to 10 before they acquire an immunity, and many of them will suffer from growth retardation, will be hospitalised several times, receive transfusions, or succumb.

Symptoms and signs are highly depen-

dent on the immune status of the patient. The transition from simple parasitemia that sustains immunity, to symptoms unequivocally attributable to disease, is not step by step, but continuous. It is difficult to set a universal clinical threshold. It depends on the species and the strain of the parasite, the parasite load and the immunity of the patient.

The age of the patients who suffer from malaria will be influenced by the endemicity of malaria in a given region. In hyperendemic ("malaria stable") countries, small children will be more affected. In hypo-endemic ("malaria unstable") regions, among displaced people and migrants, adults will be affected in a similar way to children, depending on their exposure to mosquito bites. Immunity may fade temporarily in pregnancy, and temporarily or permanently in people leaving the endemic region.

Up until now, there is no definite proof of an enhancing or a protective effect of malnutrition on the clinical severity of malaria infection (Greenwood, 1991; Van den Broeck, 1993)[1, 2]. Although iron chelation has a therapeutic effect in severe malaria (Gordeuk, 1992)[3] and iron suppletion to malnourished children can result in recrudescence of malaria infection, the protective effect of iron deficiency remains to be proved (Greenwood, 1991)[1]. Recently, the long accepted excess risk of splenectomy for malaria infection and for severity of this infection was also challenged (Looareesuwan, 1992)[4].

In recent years increased travel, transmission, endemicity and resistance of the parasite to prophylactic regimens has caused a considerable increase in imported malaria in Western countries (Bruce Chwatt, 1980; Molineaux, 1989; Steffen, 1992; Gay, 1990; Ducoffre, 1991; Freedman, 1992)[5, 6, 7, 8, 9, 10]. Since 1990 however, a decline has been reported for Caucasians, but not for Africans (Voyer, 1994; Anonymous, 1994)[11, 12]. Delayed diagnosis is responsible for avoidable morbidity and mortality (Steffen, 1992)[7]. Together with an increase in the number of cases of imported malaria, a shift towards more subacute disease has been noted (Sansonetti, 1986; Charmot, 1988; Van Gompel, 1991; Wetsteyn, 1995)[13, 14, 15, 16].

Falciparum malaria

Classical presentation

Symptoms and signs

Malaria is generally known as the disease that causes fever every two or three days. On a global scale, this description is totally false: only a small percentage of all malaria morbidity follows this pattern.

How can we explain this historical error? Malaria was first described as the disease that afflicted some regions in Europe, and later as the disease imported by expatriates. In the first case, most of the disease was caused by *P. vivax*, where relapses in fact cause fever every two days. In the second case, because of the time taken to travel by boat, expatriates were seen two or three weeks after they left the endemic region, by which time *P. falciparum* infections could present as fever every two days, reflecting the normal pattern later in the course of the disease, if spontaneous evolution is not interrupted by treatment. Finally, the periodic pattern is only seen when the infection is caused by one strain, which is not always the case in endemic areas.

The physiopathological background of malaria infection has influenced the description of symptoms and signs: since we know that malaria causes haemolysis, we readily accept that malaria infection presents with pallor, jaundice and dark-coloured urine. These signs present rather late in the course of the infection, and herald an evolution towards severe malaria. In fact, they should not be mentioned in the classical presentation.

The classical presentation can be divided into two stages: the invasive and the late phase. During the first days of disease, fever will follow an irregular pattern, almost every day. In the second phase, fever can become periodic, every two days (figure 1). During this phase, the disease can evolve towards a severe malaria, or produce a certain degree of immunity, and disappear (temporarily).

Symptoms include fever, chills, sweating, headache, arthralgia, myalgia, labial herpes, nightsweats, prostration, pain in the left subcostal region and gastrointestinal symptoms such as diarrhoea, vomiting and ab-

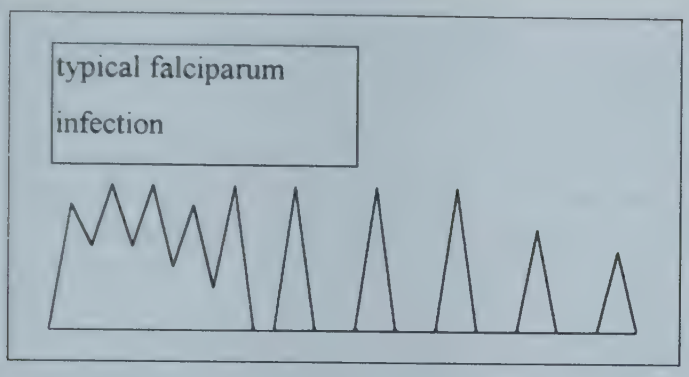

Fig. 1. Falciparum infection: during the first phase the fever is present every day, in an irregular fashion. Afterwards, fever can become tertian, presenting every second day.

dominal pain. Children may have convulsions. In the second phase, the so-called "typical malaria attack" can be seen: chills followed by high fever, resolving within a few hours with heavy perspiration, repeated every 48 hours. After the attack there may be a feeling of euphoria, but, once again, this is the exception rather than the rule.

The only reliable physical sign is splenomegaly. It develops after days or weeks. When clinically detectable pallor, jaundice and dark coloured urine develop, an evolution towards severe malaria should be suspected. Hepatomegaly is controversial: some authors give it emphasis, others do not (Manson-Barr, 1987; Gentilini, 1985)[17, 18]. Perhaps the way of confirming this sign can explain the difference in the literature: when percussion is added to the physical exam, more hepatomegaly might be found.

Of all these symptoms and signs, none is typical for malaria except the 48 hours periodicity, which is rare. Clusters of symptoms and signs are predictive in an hyperendemic region, mainly because the pretest probability is high, not because the clusters are specific (see chapter "Diagnosis of malaria disease").

Laboratory

An uncomplicated malaria attack can be accompanied by a fall in the haemoglobin level, the thrombocyte count, the total serum cholesterol and HDL cholesterol levels (Van Branden and Nelis, 1933)[19]. The leucocyte count can be affected in two ways, towards hyperleucocytosis and leucopenia.

Haemolysis parameters include a high reticulocyte count, a low haptoglobin level, a high indirect bilirubin and lactic dehydrogenase level.

Liver enzymes and direct bilirubin may be elevated; important elevations are indicative of severe malaria.

Transient albuminuria is possible in both complicated and uncomplicated *P. falciparum* malaria.

Severe and very severe malaria

Introduction

In most people, there is a fine balance between malaria infection and immunity. When this balance is disturbed and the infection becomes dominant, it can cause an acute overwhelming infection by parasites leading towards multiple organ failure (MOF), or to a subacute downhill course with progressive destruction of red blood cells. Both acute and subacute evolutions are severe and can end in death.

The world health organisation has published criteria for severe and very severe malaria: they allow an early alarm, before a frequently irretrievable situation develops (Warrel, 1990)[20]. Table 1 lists defining criteria along with manifestations that are common, but not necessarily indicative of severe malaria. A practical consequence of the diagnosis of severe malaria is the need for parenteral treatment and hence for referral. Severe vomiting is not a defining criterion of severe malaria, but has the same consequence, and is therefore often included in the defining list.

Severe malaria is life-threatening, but treatment is lifesaving: in a spectacular case of cerebral malaria with 70% of RBC infected, the patient was resuscitated, he developed aspiration pneumonia, ARDS, renal failure, sepsis, peritonitis and splenic necrosis, but he survived with exchange transfusion (Files, 1984)[21]. Another patient with 71% of RBC infected survived without exchange transfusion (Marik, 1989)[22].

Case report ([1])

A. S., a 33-years-old seaman from Cape Verde, was hospitalised after a recent jour-

([1]) This case has been published before (Van den Ende, 1994)[23].

Defining criteria
• unarousable coma
• severe normocytic anaemia (haematocrit < 15% or haemoglobin < 5 g/dl)
• renal failure (creatinine > 3 mg/dl and urine output < 400 ml/24 h in adults, <12 ml/kg/24 h for children)
• pulmonary oedema
• hypoglycaemia (whole blood glucose concentration < 40 mg/dl)
• circulatory collapse, shock
• spontaneous bleeding/disseminated intravascular coagulation
• repeated generalised convulsions (> 2 convulsions/24 h despite cooling)
• acidosis
• malarial haemoglobinuria
Other manifestations
• impaired consciousness, but rousable
• prostration, extreme weakness
• hyperparasitemia (> 5% infected RBC or > 250.000 parasites/ml)
• jaundice (serum bilirubin concentration > 3 g/dl)
• hyperpyrexia

Table 1. Criteria for severe *Pl. falciparum malaria*.

ney to West-Africa, with fever, diminished consciousness, jaundice and oliguria. No prophylactic drugs had been taken. Laboratory results included the presence of Plasmodium falciparum in both thick film and blood smear with 25% of RBC parasitized, haemoglobin of 5.12 g/dl, thrombocyte count of 28 x 10^9/mm, total serum bilirubin of 8.06 mg/dl, serum creatinine of 8.1 mg/dl and a plasma free haemoglobin level of 55,6 mg/dl. Plasma glucose and ions were normal.

Quinine, 500 mg IV t.i.d. was started immediately. Given the high parasitemia and the clinical condition, an exchange-transfusion with 4l of reconstituted blood was begun. At the end of the exchange transfusion the parasitemia was 2%.

On the first and the fourth day, an additional transfusion of 2 respectively 3 units of packed-cells was given. Haemodialysis was performed on the first, third and fourth day. After the first 24 hours, administration of quinine was restricted to once a day, and an additional dose after each hemodialysis, until kidney function improved. Measurement of serum quinine level showed a concentration of 3 microgr/ml.

By the end of the exchange transfusion, consciousness returned to normal, and on the third day 2086 ml of urine was produced. The patient was discharged after complete recovery.

Clinical picture

An evolution towards an acute MOF is possible after a first infection. If untreated, mortality in an nonimmune patient can be as high as 20% (Fairley, 1945)[24]. This evolution can also appear in the course of building up immunity, or superimpose on a subacute severe evolution. It is rare or non-existent in semi-immune older children and adults in endemic regions.

Often several organs are affected at the same time but exceptions are frequent, in which e.g. only cerebral malaria develops and where kidneys, liver and lungs are relatively unaffected (Weber, 1991)[25]. Manifestations of lung involvement may be late, although early changes can be seen radiographically.

a) Cerebral

Cerebral malaria generally presents as

an acute diffuse encephalopathy. Cerebral oedema and foramen magnum herniation are rare (Looareesuwan, 1995)[26]. Convulsions are frequent in children with fever. An isolated convulsion does not establish the diagnosis of cerebral malaria, but it constitutes a serious warning. In adults, it unequivocally heralds a cerebral involvement. Repeated convulsions, or status epilepticus are defining criteria of cerebral malaria. Convulsions are mostly generalised, but Jacksonian convulsions or widespread myoclonias are possible (Daroff 1967; Geerts 1995)[27, 28].

Altered consciousness is often the first sign of severe malaria, but can be provoked by high fever itself. Disorientation in time and space, amnesia, difficulty with speech can progress to drowsiness, ending in coma. This evolution can be very fast, a matter of a few hours.

Acute signs of focal affection of the brain (paralysis, cranial nerve dysfunctions, cortical blindness...) are relatively rare in adults, but not in children. These focal signs may persist as sequelae in up to 10% of survivors, especially when the cerebral malaria is complicated by hypoglycaemia (Taylor T. E., 1988; Schmutzhard E., 1984)[29, 30]. Acute and delayed cerebellar dysfunction with hypotonia and nystagmus have also been described.

Neck stiffness has been described as one of the presentations of cerebral malaria. It may be part of an opisthotonus or of a decerebration rigidity. Lumbar puncture yields a normal fluid. Meningitis and other locally common causes of encephalitis should be excluded.

Retinal haemorrhages may occur, exudates and papilloedema are rare. Pupils are normal, as are corneal and eyelash reflexes, but disorders of gaze are frequent.

Tendon reflexes are generally increased, but abdominal reflexes are absent, which may help in excluding a hysterical crisis in a feverish patient.

b) Liver

Some hepatocellular necrosis is common in all forms of malaria. In severe malaria, hepatic enzymes may rise severely (but never as high as in viral hepatitis), and direct bilirubin can contribute substantially to the total bilirubin elevation. Liver-related symptoms are rare; frank jaundice is possible, and reflects both haemolysis and liver dysfunction. Clinical signs and/or liver failure are never seen. The production of coagulation factors can be affected, with prolonged prothrombin time. Hepatic dysfunction may contribute to low serum albumin, hypoglycaemia and lactic acidosis.

c) Kidneys

The pattern of the kidney dysfunction is that of an ischaemic nephropathy or an acute tubular necrosis. Adults are much more subject to this complication than children; in African children it is uncommon (White 1988; Molyneux 1989)[31, 32]. The symptoms include oliguria, anuria, and occasionally polyuria. Dark coloured urine is due to concentration and haemoglobinuria. Urea and creatinine rise, despite correct rehydration and hemodialysis or peritoneal dialysis is often necessary. During recovery, which can take several weeks, a polyuric phase is possible.

d) Lungs

The exact pathogenetic mechanism of lung involvement in severe malaria is not known. It may be explained by increased pulmonary vascular resistance during severe falciparum malaria infection, hypoalbuminemia and volume overload caused by infusions (Poonkasem Charoenpan, 1990; Feldman 1987; Tatke 1990; Pradeep Bambery, 1991)[33, 34, 35, 36].

Too often, pulmonary dysfunction is provoked or increased by the rapid administration of large amounts of fluid, with the aim of reversing oliguria or anuria, based on the idea that tubular haemoglobin might be responsible for renal function impairment. However, cases also occur with a normal or negative fluid-balance.

The classical clinical picture is one of pulmonary oedema or ARDS, with cough, dyspnoea, polypnea and cyanosis. This complication has a high mortality, and often comes late in the course of the severe malaria, sometimes in patients recovering from other manifestations of the disease (Brooks, 1968)[37]. Polypnoea can also be provoked by acidosis, but auscultation should allow the distinction between the two causes, with inspiratory crackles and

Fig. 2. ARDS as a feature of severe malaria in a patient presenting with pulmonary oedema, cough, dyspnoea, polypnea and cyanosis.

rales, together with X-ray images, which can be quite impressive, ranging from an interstitial overload to white lungs (figure 2). Blood gas analysis shows low oxygen content and saturation.

Recently, development of ARDS as a direct consequence of the procedure of exchange transfusion has been challenged (Vachon, 1990)[38]. In the series of Miller et al., six out of twelve patients developed this complication (Miller, 1989)[39].

e) Gastro-intestinal

Nausea, vomiting and abdominal pain are common symptoms. Severe diarrhoea and even choleraic diarrhoea have been described with hyperparasitemia, leading to dehydration and hypotension. The diarrhoea resolves with treatment of malaria alone.

f) Cardiovascular

Hypotension is common with dehydration, pulmonary oedema, acidosis and septicaemia as contributory factors. In spite of a high parasite load in the capillaries of the heart, cardiac manifestations such as heart failure or arrhythmias are rare. Diffuse intravascular coagulation can

Fig. 3. Diffuse intravascular coagulation leading to vascular occlusion and necrosis of toes.

lead to vascular occlusion, and cause necrosis of fingers and toes (figure 3).

g) Blood

High parasitemia is common, and the number of parasitised red blood cells can exceed 50% of red blood cells parasitised (Files J. C., 1984; Marik, 1989)[21, 22]. Mortality is to some extent related to parasitemia (Warrel, 1987; Warrel 1989)[40, 41]. Some authors describe cases of cerebral malaria with no parasitemia, (Warrel 1990; Chia 1992; Commey, 1984)[20, 42, 43] while others discuss this clinical entity, and emphasise its rarity (White 1992)[44].

Low haemoglobin levels and thrombocyte counts are common. Leucocyte counts can be normal, low or high. A high leucocyte count reflects clinical severity (Warrell, 1982; Molyneux, 1989)[45, 32]. Very low thrombocyte counts ($< 10 \times 10^9$/l) can be observed without bleeding.

Acidosis is related to shock, liver dysfunction, renal failure and hypoglycaemia. It can be pronounced, and can lead to polypnea. Mild hyponatremia is common but profound hyponatremia which can cause coma, is rare. Diffuse intravascular coagulation is rare but it can cause low thrombocyte counts and the presence of fibrin dimers in serum and urine. Other findings are hypocalcemia, hypophosphatemia, hyperphospholipidemia, hypertriglyceridemia, hypocholesterolemia, and the sick euthyroid syndrome with low total serum thyroxine concentration and normal TSH. These findings are clinically not relevant.

h) Hypoglycaemia

Hypoglycaemia is frequent in severe malaria in children in hyperendemic regions (Warrell 1990; Phillips 1990; Marsh 1995; Mabeza 1995)[20, 46, 47, 48]. It can contribute to clinical severity and to late sequelae. It is a predictor of mortality.

Hyperinsulinemia provoked by quinine treatment is one of the suggested causes of hypoglycaemia but in African children no correlation between hypoglycaemia and quinine therapy was found (White, 1987; Taylor, 1988)[49, 29]. Hyperparasitemia itself or the combination of malaria and pregnancy can also be a cause without quinine therapy. Hypoglycaemia is not restricted to severe malaria, but has also been described in other life-threatening conditions in children in poor countries (Kawo, 1990)[50].

Often hypoglycaemia is not clinically recognisable: it contributes to coma, but may not produce the classical symptoms of sweating, dizziness, tachycardia etc. No one clinical sign is reliably indicative of hypoglycaemia.

i) Septicaemia

Gram negative septicaemia often occurs with very severe malaria, even in the absence of a focus such as an indwelling catheter or a decubitus ulcer. If hypotension is present, septicaemia should always be suspected, and treatment should begin without waiting for the results of hemocultures.

Hyperreactive malarial splenomegaly (HMS)

Introduction

The tropical splenomegaly syndrome (TSS) originally referred to cases of splenomegaly in the tropics for which no cause was found despite thorough investigation (De Cock, 1983)[51]. Many early investigators applied the term "tropical splenomegaly" to syndromes representing probably non-cirrhotic portal fibrosis (Williams, 1966; Hewlet, 1987)[52, 53], or visceral leishmaniasis (Hewlet, 1987)[53]. Since 1976 TSS refers specifically to "a condition resulting from an aberrant immunological response to malaria" (Marsden, 1976)[54]. In 1981 Fakunle set major and minor diagnostic criteria (Fakunle, 1981)[55] (Table 2). Splenomegaly, elevated antimalarial anti-

***Major diagnostic criteria*: always present**
• gross splenomegaly in older children and adults
• high antibody levels for *P. falciparum*
• elevated serum (polyclonal) IgM of at least 2 standard deviations above the mean of the population from which the patient comes
• clinical and immunological response to long term *appropriate* antimalarial therapy, but no reduction in spleen size may be apparent for the first three months
***Minor diagnostic criteria*: frequently or nearly always present**
• hepatic sinusoidal lymphocytosis is a diagnostic histologic finding, occurring in over 80% of cases (only seen in Felty's syndrome, non-tropical idiopathic splenomegaly or Dacie's syndrome, and some rare cases of chronic lymphocytic leukaemia)
• normal cellular and humoral immune responses to antigenic challenge (except to malarial antigens, where enhanced lymphocyte proliferation has been shown)
• normal response to PHA
• hypersplenism
• lymphocyte proliferation (in some populations)
• occurrence within families, tribes

Table 2. Diagnostic criteria of hyperreactive malarial splenomegaly.

bodies, elevated IgM and response to long term treatment or prophylaxis are major criteria. Hepatic sinusoidal lymphocytosis, occurring in over 80% of cases (Marsh, 1986)[56], and hypersplenism are minor criteria. In 1983 an international group of workers interested in the problem of splenomegaly in the tropics proposed replacing the name "tropical splenomegaly syndrome" by "hyperreactive malarial splenomegaly" (HMS) in order to distinguish between splenomegaly of obscure origin and splenomegaly related to malaria (Bryceson, 1983)[57].

It is not uncommon to find cold agglutinins amongst the non-specific, "wild", IgM antibodies. These cold agglutinins can further exacerbate haemolysis. The splenomegaly by itself can cause hypersplenism, in which not only erythrocytes, but also leucocytes and thrombocytes will be destroyed or removed from the circulation. However, this hypersplenism is not a pre-requisite for the syndrome.

HMS has only rarely been reported in Caucasians. HMS can develop years after a stay in an endemic region: in one case, it developed 8 years after the last stay in a tropical country (Torres-Rojas, 1981)[58].

Long term exposure to malaria over a period of five years or more is necessary for the development of this syndrome which only affects adults and older children.

Case report ([1])

V. H., a Dutch boy, was born in north-eastern Zaire where he lived until the age of 6. He had been taking chloroquine, 5 mg/kg twice a week as malaria prophylaxis. He had a normal development and never experienced any serious health problem in Zaire. Eighteen months after return, he became ill (December 1987). The parents noticed pallor, weight loss, asthenia and diminished performance in sports. The boy had two short episodes of fever with headache. He was treated empirically by the parents with chloroquine (25 mg/kg, in three days). They reported slight improvement. A paediatrician found an impressive splenomegaly. A sonographist reported a maximum spleen diameter of 17 cm. A thick film did not reveal plasmodium parasites, but serum antibodies to P. fieldi (a laboratory plasmodium strain that shares antigenic properties with P. falciparum) were 1/320 in immunofluorescence.

The boy was referred to the outpatient department of the Institute of Tropical Medicine, Antwerp, where he was seen on April 15, 1988. Asthenia and weight loss were still present. Clinical examination revealed a pale face, with normally coloured conjunctivae, and a spleen that descended 4 fingers under the costal margin. Haemoglobin was 11,2 g/dl, leucocyte count 7.5 x 10^9/l with normal formula, thrombocyte count 188 x 10^9/l, reticulocyte count 6.4%, sedimentation rate 39 mm/h, and haptoglobin 0 mg/dl. A thick film was negative for parasites. A Knott concentration technique revealed microfilaria of Dipetalonema (Mansonella) perstans. Schistosoma infection was excluded by negative stool examination (direct and after concentration), and by negative serology (ELISA, enzyme linked immunosorbent assay). P. falciparum antibodies were 1/5120 in indirect immunofluorescence (IFAT, Ig-class aspecific). Antibodies for P. vivax, P. ovale and P. malariae were 1/320, 1/640 and 1/320. Gammaglobulin fraction was 25,9% or 2,04 g/dl; IgM content was 1090 mg/dl (normal values for age 6-8 y: 27-118 mg/dl (Stiem, 1966)[60]. Osmotic resistance of erythrocytes gave a normal curve, except for complete haemolysis which required 0,100 g/dl.

Ultrasonography of the upper abdomen showed a normal appearance of the liver, gallbladder, kidneys and pancreas. The maximal spleen diameter was 13,5 cm.

The patient was treated with mefloquine, 30 mg/kg in divided doses over 24 hours. His complaints disappeared, and he regained weight.

The evolution of spleen size and laboratory parameters is shown in Table 3. All laboratory parameters responded to the first treatment. For the haptoglobin, IgM and P. falciparum antibodies it took several months to regain normal values.

Given the persistence of the splenomegaly and the extremely low haptoglobin, a second identical treatment with mefloquine, followed by weekly mefloquine, 6 mg/kg/week for 8 weeks was prescribed, 8 months after the

([2]) This case has been published elsewhere (Van den Ende, 1994 annales)[59].

Date	15/04/88	13/05/88	16/06/88	4/08/88	13/01/89	20/10/89	11/04/90	01/06/92
Hb	11,2	12,2	12,4	12,5				
Ret	64	25	16	13				
Sed. rate	39	21	12					
Hapto	0	0	0	0	77	62	37	85
IFAT	5120	1280	1280	1280	640	1280	320	20
Gamma	2,04	1,76	1,59	1,39				
IgM	1090	717	520	341			177	177
Spleen size	13.5	14	14	14	14	14	14	13
Height	118							156

Hb: haemoglobin in g/dl.
Ret: reticulocyte count per 1000 erythrocytes.
Sed. rate: sedimentation rate (mm/h).
Hapto: haptoglobin in mg/dl.
IFAT: anti *P. falciparum* antibodies in immunofluorescence (dilutions)
Gamma: gammaglobulin fraction in g/dl.
IgM content in mg/dl.
Spleen size in cm maximal diameter.
Body height in cm.

Table 3. Evolution of clinical parameters of the HMS case.

first treatment. No effect on splenomegaly was noticed.

During the next 4 years all laboratory parameters normalised, and a normal development was noticed. The spleen did not decrease in size, but became normal for age as height increased.

Clinical picture

This syndrome may be relatively asymptomatic (Hewlet, 1987)[53], but pallor, weight loss, asthenia, diminished performance in sports, dyspnoea on exertion, pain and heaviness in the left subcostal region can occur, as can short episodes of fever.

Pale conjunctivae and tachycardia signal anaemia. Splenomegaly is always found on physical examination and can be confirmed by ultrasound but no typical changes in the spleen or in the liver will be seen.

A thick film will generally not reveal plasmodium parasites. Serum antibodies to *P. falciparum* (indirect immunofluorescence, IFAT, where available) will be extremely high. Cross immunity to *P. vivax*, *P. ovale* and *P. malariae* is also common.

A low haemoglobin level, leucocyte count (with normal formula) and thrombocyte count reflect hypersplenism. A low serum haptoglobin level, a high reticulocyte count and serum lactic dehydrogenase level are signs of haemolysis. The sedimentation rate, the serum gammaglobulin fraction and the serum IgM content reflect a wild antibody response (Stiem, 1966)[60]. The osmotic resistance of erythrocytes gives a normal curve.

Cold agglutinins can cause quick agglutination when taking a blood slide (this phenomenon has nothing to do with coagulation). In some cases, a rapid "sedimentation", within a few minutes, can be observed in blood collecting tubes. Again, this is agglutination, not sedimentation.

One of the criteria for diagnosis is the evolution of spleen size and laboratory parameters with treatment. Reduction in spleen size can be very slow (Fakunle 1981, Hewlett 1987)[55, 53].

Liver biopsy can show hepatic sinusoidal lymphocytosis. Given its intrinsic morbidity, it should not be performed routinely. In most cases, one can obtain

enough clinical evidence for HMS by ruling out other causes of splenomegaly and observing the improvement of the clinical condition during antimalaria treatment. Moreover, although hepatic sinusoidal lymphocytosis is often referred to as a pathognomonic sign, it is also seen in Felty's syndrome, non-tropical idiopathic splenomegaly or Dacie's syndrome, and some rare cases of chronic lymphocytic leukaemia (Crane, 1986)[61].

Subacute malaria

Introduction

Between acute malaria and HMS we find an array of ill-defined syndromes of subacute malaria. When a first infection is not adequately treated, partial immunity may develop, and a certain morbidity may follow. An overwhelming infection in a semi-immune patient, where the delicate balance between immunity and parasite is ruptured, can have the same consequences. This is certainly the case in a lot of children in endemic countries, who will develop an increasing anaemia, even without bouts of fever (Greenwood, 1987)[62]. The same is the case in pregnant women, who are particularly vulnerable to subacute disease, with repercussions for the foetus.

In adults in endemic countries, the balance clearly favours immunity, they will develop malaria attacks from time to time but subacute morbidity is rare. For migrants and expatriates, the situation is different, and can be compared with the situation of children in endemic countries. (For the clinical picture in children and pregnant women we refer to chapter "Clinical features of malaria in pregnancy and children").

In recent years subacute malaria has become also more frequent in travellers and residents after return from malarious countries. The emergence of chloroquine-resistant *P. falciparum* strains, and the partial suppression of the infection by ineffective prophylaxis or treatment have been blamed for this increase (Charmot, 1988; Sansonetti, 1986; Van Gompel 1991; Wetsteyn, 1995)[14, 13, 15, 16]. However, prophylaxis use or inadequate treatment are not absolute prerequisites (Van den Ende, unpublished data).

There are no strict criteria which can delineate subacute malaria from HMS, except for spleen size. Patients with subacute malaria develop high plasmodium antibody titres and high serum IgM levels; some will develop frank HMS, but some other patients fulfil all major criteria for HMS, except for the splenomegaly.

The overall impact of subacute malaria on general health in endemic regions is not well known, and will be discussed in chapter "Diagnosis of malaria disease".

Case report

A 62-year-old Belgian missionary, living in Zaire since 1957, had a medical check-up in 1984, without complaints. An anti-P. falciparum IFAT titer of > 1/640 was detected. Malaria prophylaxis was proposed, but not taken. In 1986 he presented with diarrhoea, vomiting and weight loss of 8 kg. A thorough work-up could not elicit an aetiology for the diarrhoea. Sedimentation rate was 81 mm after 1h, in the thick film rare trophozoites of P. falciparum were reported, malaria antibodies again were higher than 1/640 and serum IgM content was 1295 mg/dl. Malaria was treated. The patient spent six months in Belgium, and the sedimentation rate dropped to 21 mm/1h.

In Zaire he again neglected to take malaria prophylaxis. He returned to Belgium in 1989 with diarrhoea, fatigue and weight loss. Physical examination was negative. The thick film was negative, the sedimentation rate was 63 mm after 1h, the malaria antibodies 1/5120, and the serum IgM 1720 mg/dl. Antinuclear antibodies were 1/80. Circulating immune complexes were detected. Maximum sonographically measured diameter of the spleen was 15 cm. Again, no cause for the diarrhoea was detected. After an initial treatment with quinine and doxycycline, mefloquine (1500 mg in one day) was added because laboratory parameters did not improve. After two months, all symptoms had disappeared, including the diarrhoea. The sedimentation rate had dropped to 35 mm/1h and the IgM to 910 mg/dl. Prophylaxis with chloroquine 300 mg once a week, and proguanil, 200 mg/day was prescribed.

This time, prophylaxis was taken regular-

ly, but in 1992 diarrhoea and weight-loss occurred again. Physical examination was negative. Haemoglobin was 11.8 mg/dl and the sedimentation rate 102 mm/1h; a thick film showed 4 trophozoites of P. falciparum per 100 microscopic fields (10 x 100), malarial antibodies ranked as high as 1/10240, IgM was 2680 mg/dl. Anticytoplasmatic antibodies were 1/5000 in a speckled pattern. The spleen was not enlarged sonographically. A one day treatment of halofantrine (1500 mg) was prescribed. The patient remained in Belgium. After six months he was symptom free, the sedimentation rate was 38 mm/1h, the haptoglobin 112 mg/dl, the anticytoplasmatic antibodies 1/640, the malarial antibodies 1/10240 and the IgM 925 mg/dl.

Clinical picture

In expatriates and migrants, episodes of fever, nightsweats, pallor, weight loss, asthenia, dyspnoea on exertion, pain and heaviness in the left subcostal region have been reported. On physical examination pale conjunctivae and splenomegaly can be found. In a minority of cases repeated thick films can confirm the diagnosis, even in the absence of fever. Serum antibodies to *P. falciparum* are high.

A low haemoglobin level, leucocyte count (with normal formula) and thrombocyte count are generally present. A low serum haptoglobin level, a high reticulocyte count and a high serum lactic dehydrogenase and bilirubin level show haemolysis. The sedimentation rate, the serum gammaglobulin fraction and the serum IgM content are elevated. Cholesterol and HDL-cholesterol are low (Cuisinier-Raynal, 1990)[63]. As in HMS, cold agglutinins may be present.

Black water fever (BWF)

Introduction

Numerous definitions or descriptions of BWF and its cause have been proposed and discussed by various authors (James, 1922; Findlay, 1949; Ross, 1962; Bruce-Chwatt, 1980; Knochel, 1993)[64, 65, 66, 5, 67]. There are widespread descriptions of cases with fever, jaundice and haemoglobinuria or passing black urine throughout history of medicine, dating as far back as Hippocrates. Case reporting started mainly around 1820; by 1850 cases were reported from almost all continents. BWF has been related to *P. falciparum* infections since 1920 (Plehn, 1920)[68]. BWF has even been recorded in induced plasmodium infections for the treatment of syphilitics (James, 1922)[64]. It is likely that many cases of BWF in the past were caused by other diseases or conditions, such as glucose-6-phosphate dehydrogenase deficiency (G-6-PD) or leptospirosis.

BWF is apparently triggered by antimalarials. The possible etiologic role of quinine was recognised in 1937 (Stephens, 1937)[69]. It is not clear if the haemolysis is purely drug-induced, or if there is another physiopathological link with antimalarials. Until now, the exact pathogenic role of the parasite and the antimalarial drugs has not been clarified. In BWF the direct Coombs test is negative, whereas in a classical drug-induced haemolysis the Coombs test is unequivocally positive (Dacie, 1991)[70].

Recently mefloquine and halofantrine have been added as possible triggers, although a classical drug-induced haemolysis was not excluded in these cases (Danis, 1993; Vachon, 1992; Mojon, 1994)[71, 72, 73]. The triggering by mefloquine and halofantrine may not be so surprising since the three amino-alcohols have rather similar structures (see figures 6 and 7, chapter on antimalarial drugs by Wernsdorfer), and cross-resistance has been shown in vitro between amino-alcohols (Peel, 1994)[74]. Recently two cases of successive BWF syndromes have been described, where the first attack was triggered by quinine, the second by halofantrin (Van den Ende, unpublished data).

The absence of BWF from the literature for decades is interesting. There are two possible explanations:

- after the world war II the campaign for the global eradication of malaria resulted in a temporary and relative control of malaria endemicity. The decreased efforts for malaria control in many areas of the world, the resistance of mosquitoes to DDT, and of the parasite to chloroquine, provoked a worldwide revival of malaria. Perhaps we see more BWF because we see more malaria;

- in that period quinine was restricted to seriously ill patients. The widespread advent of chloroquine resistance made treatment with quinine or newer antimalarials such as mefloquine and halofantrine more popular, also as presumptive treatment for minor symptoms.

Case report

A 64-year-old Belgian catholic sister, working in Mali, did not take any prophylactic medication for malaria, but for two years she treated bouts of fever or fatigue with small doses of quinine, aware of parasite resistance to chloroquine.

On the second of October 1990 she complained of vomiting, fatigue and anorexia. Quinine was started at a rate of 2 x 500 mg/day. The third of October she reported red urine, fever up to 38.5°C and an incipient jaundice. The following day there was frank jaundice, fever, nausea and dark urine. A thick film showed very rare trophozoites of P. malariae (diagnosis in Mali, blood smear not available for confirmation). Laboratory abnormalities included : haemoglobin 6.2 g/dl, sedimentation rate 145 mm after one hour, urea 83.4 mg/dl, serum creatinine 0.64 mg/dl, alkaline phosphatase 95 U/l (nl 21-92), conjugated bilirubine 3.19 mg/dl, non-conjugated bilirubin 5.6 mg/dl, ALT (alanine amino transferase) 125 U/ml (nl < 40), AST (aspartate amino transferase) 145 U/l (nl < 45). Intravenous liquids, 4 x 500 mg quinine IM and 3 x 300 mg chloroquine orally were administered before repatriation.

On the 6th of October, the patient arrived at the Antwerp University Hospital. She was very pale, and dyspnoeic with the slightest exertion. The temperature was 38° C, auscultation revealed a few faint crackles, the liver extended 2 cm under the costal margin, the spleen was not felt.

Key laboratory tests: Haemoglobin 4.9 g/dl, lactate dehydrogenase 2040, total bilirubin 3.5, AST and ALT normal, haemoglobinuria (27 mg/l), elevated urea (113 mg/dl) and creatinine (1.8 mg/dl). A thick film for malaria was negative, cultures of blood and urine remained sterile.

Further work-up for haemolysis: cold agglutinins anti-I : 1/32 at 4°C, osmotic resistance slightly lowered; HAM test, polyvalent RAGT test, sucroselyse and Donath-Landsteiner negative. Vitamin B 12, folic acid and glucose-6-phosphate dehydrogenase were within normal range.

Serology: hepatitis B surface antigen negative, hepatitis A IgM antibodies negative, Mycoplasma pneumoniae antibodies 1/100, HIV antibodies negative, antibodies for P. falciparum 1/10240 in indirect immunofluorescence (IFAT). A chest X-ray was normal. An abdominal ultrasound showed hepatosplenomegaly, thickening of the gallbladder wall, and a few small lymph nodes in the porta hepatis. An echocardiographic examination showed some thickening of the aortic valve without vegetations.

Four units of packed cells, halofantrine (3 x 500 mg/12 h, once) and subcutaneous heparin as thrombosis prevention were administered. One month later, she was symptom free. Haemoglobin was 12 mg/dl, and the sedimentation rate was 110 mm/h. Two months later the sedimentation rate had dropped to 68 mm/1h, and the IFAT to 1/5120. She returned to Africa with 300 mg of chloroquine a week and 200 mg of proguanil a day as malaria prophylaxis.

Clinical picture (Table 4)

A diagnosis of BWF should be considered in a person living in a malarious area if there is haemoglobinuria, a dramatic fall in haemoglobin, and a thick film showing no or very few parasites.

In most cases it is possible to trace a history of successive malarial attacks, often inadequately treated (Maegraith, 1948)[75]. In most cases, BWF follows the intake of quinine, mefloquine or halofantrine. Massive haemolysis in one or several waves is common: several million RBC /mm3 can be destroyed in 24 hrs. The urine is often red at first and afterwards dark-brown. Blood urea nitrogen is elevated, overt renal failure can be present. Sometimes a polyuric phase follows recovery from anuria. Acute epigastric discomfort, nausea and vomiting can be present. Jaundice was present in 33 of 46 (71%) fatal cases of BWF in the British West African Colonies in the period 1941-43 (Findlay, 1949)[65]. Liver function abnormalities appear early in many cases. Vascular col-

Key features
• exposure to malaria
• massive haemolysis
• haemoglobinuria
• no or scanty parasites on thick film
Additional characteristics
• history of successive malarial attacks, often inadequately treated
• intake of quinine, mefloquine or halofantrine
• epigastric discomfort, nausea and vomiting
• jaundice
• elevated blood urea nitrogen, renal failure
• polyuric phase during recovery
• liver function disturbances
• vascular collapse

Table 4. Symptoms and signs of BWF.

lapse is possible, followed by death in 20 to 30 % of patients (Bruce-Chwatt, 1980)[5]. Methaemalbuminemia is frequent and representative of the severity of the haemolysis (Dacie, 1991; Tietz, 1990)[70, 76].

Blackwater fever should be distinguished from very severe malaria with hyperparasitemia, where massive haemolysis, liver and renal failure contribute to jaundice. Other causes of intravascular haemolysis must be excluded.

Glomerulonephritis

Transient albuminuria is possible in both complicated and uncomplicated *P. falciparum* malaria. In rare cases a frank glomerulonephritis may develop. Plasmodial antigen and antiplasmodial antibodies in glomerular deposits can be demonstrated (Sinniah, 1988; Van Velthuysen, 1996)[77, 78].

Albuminuria, the presence of casts in urine, and a nephrotic syndrome with oedema of the face and ankles and ascites are the main clinical features. Hypertension is absent. In contrast with *P. malariae* renal involvement, this picture never evolves towards chronic glomerulonephritis and renal failure. Treatment with antimalarials alone is 100% effective.

Spontaneous splenic rupture

Introduction

This complication is not confined to *P. falciparum* alone: it has also been reported in infections with other types of malaria. Rupture can happen during the early or late phase. The enlarged spleen is very vulnerable, and the slightest abdominal trauma or shock can be sufficient for rupture. It is a real pitfall for clinicians, as it can happen outside fever attacks, and without trauma.

Case report

On one of my (JVdE) supervision tours in Zaire in 1984, I was called to a 24-year-old woman, who was 7 months pregnant. Pregnancy was well supervised, and no abnormal finding had been reported. A weekly malaria prophylaxis with 300 mg of chloroquine had been administered. She presented with diaphoresis and abdominal pain. There was no vaginal bleeding or discharge. There was no history of trauma. Physical examination revealed a blood pressure of 70/40, a heart rate of 110/min, a negative abdominal palpation, a uterus height corresponding to 7 months, and a negative vaginal exploration. Ultrasound was not available and emergency evacuation was impossible given the distance and the extremely bad roads. Facing a dramatic clinical situation, we decided to operate at the health centre. On opening we discovered a large amount of free blood in the abdominal cavity, and with further exploration we found a transversely ruptured spleen. A splenectomy was unavoidable. Foetal heart sounds were normal at the end of the operation, so no cesarean section was carried out. At dawn however, the patient had a still-birth. Pneumococcal vaccine was not available, but the patient was put on life-long malaria prophylaxis.

Clinical picture

Abdominal pain, hypotension and transpiration may be the only symptoms. Ab-

dominal guarding is not always present, and the ruptured spleen can be missed on palpation. In the first hours, laboratory tests are useless, since haemoglobin levels are not yet adjusted by haemodilution. Abdominal ultrasound or abdominal puncture can confirm the diagnosis of this complication, but in field conditions only an exploratory laparatomy can reveal the true diagnosis.

Vivax malaria

Introduction

Vivax malaria clinical presentation is different from *P. falciparum* infections in several respects. Its incubation time is longer, relapses from hepatic hypnozoïtes are frequent, tertian fever pattern is frequent, strain type influences clinical findings, and it never leads to very severe malaria.

The WHO defined three strain types: type I has a short incubation, and frequent relapses; type II has the same short incubation and few relapses; type III has a long incubation time and few relapses.

Case report ([1])

In October 1992, a 24 year-old Belgian woman travelled to Calcutta, India. She meticulously took chloroquine 300 mg once a week as malaria prophylaxis. Two weeks after arrival in India she developed fever. Without parasitological diagnosis, chloroquine was given, and the fever disappeared. After she returned to Belgium in December 1992, she experienced several minor episodes of fever which remained untreated. In June 1993 she developed high fever and chills every second day. A thick smear showed P. vivax (1 parasite/high power field 10 x 100). Blood cell counts and biochemical parameters were normal. Chloroquine was given for 3 days (900-300-300 mg), followed by primaquine 15 mg/d for 14 days. The glucose-6-phosphate dehydrogenase level was 2.1 U/ml (normal 1-2.1).

In August 1993 she again developed high fever. Smears were again positive for P. vivax (5 parasites/microscopic field 10 x 100: 72% trophozoites, 28% gametocytes). With chloroquine (900-300-300 mg) the fever disappeared on the fourth day. General malaise and night sweats persisted. Eight days after initiating treatment, the fever returned and a thick smear showed the same parasitemia for P. vivax (5 parasites/high power field, 93% trophozoites). Oral quininesulphate (3 x 500 mg/d) was given initially, but was substituted by intravenous quininehydrochloride (3 x 500 mg/d for 5 days), since the fever did not drop after 48 hours. The quinine was combined with oral doxycycline 200 mg the first day and 100 mg/d for one week. The fever disappeared and the thick smear became negative. She returned home with primaquine, 22,5 mg/d for 15 days.

On the 12th day of primaquine treatment, the fever reappeared. Again smears were positive for P. vivax (4 parasites/high power field). Treatment with oral quinine sulphate and doxycycline was repeated. Since higher doses of primaquine often provoke serious side-effects (5), the patient favoured a prophylactic regimen of mefloquine 250 mg/week. No further relapses occurred.

Clinical picture

The incubation time is longer than for *P. falciparum* infection, and depends on the strains: currently 12 to 20 days, for some strains it may be 6 to 9 months. During the first days of disease, as in *P. falciparum* infection, fever will follow an irregular pattern, almost every day. There is nothing to differentiate a *P. falciparum* early infection from a *P. vivax* infection: fever, chills, perspiration, headache, arthralgia, myalgia, nightsweats, prostration, pain in the left subcostal region, dark coloured urine, labial herpes, can all be present. Gastrointestinal symptoms complete the picture. In children there may be febrile convulsions. Without treatment, fever disappears after some to several irregular attacks. This does not mean that the infection has disappeared, since hypnozoïtes may survive in the liver, and cause relapses.

Splenomegaly is the only physical sign. Clinically detectable pallor and jaundice

([1]) Already published elsewhere (Van den Abbeele, 1995)[79].

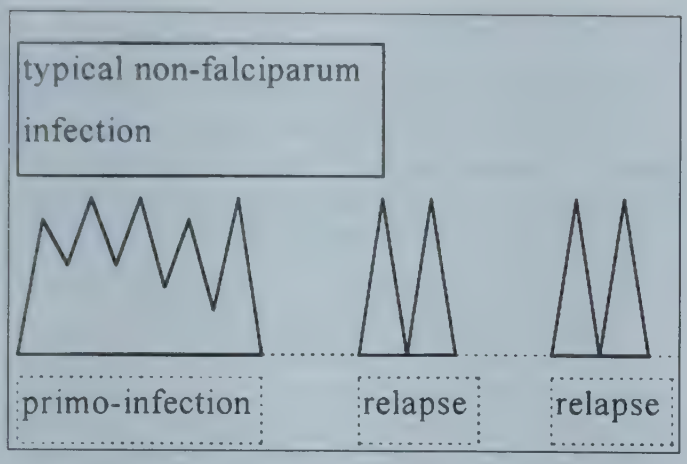

Fig. 4. In non-falciparum infection, fever is irregular during the primo-infection. During relapses, which emerge from the liver for vivax and ovale infection, and from the blood in malariae infection, the fever will follow a certain periodicity: tertian with vivax and ovale, quartan with malariae.

are rare, and are seen in the evolution towards the more subacute form, which is seen in immunocompromised people or during pregnancy.

In *P. vivax* infection, relapses cause fever every two days ("benign tertian fever") (figure 4). The periodic pattern is seen only when the patient is infected by one strain; when infected by two strains, a "double tertian fever" may result, with a fever spike every day. In relapses "typical malaria attacks" are the rule: chills followed by high fever, resolving within a few hours with heavy sweating and a feeling of relative euphoria. Intervals between relapses depend on the strain. Chloroquin treatment has a rapid effect on the symptoms, but has no effect on future relapses.

In heavily infected patients, or where there is immunodepression as during pregnancy, a severe subacute form can follow the early phase ([1]). Slight fever with irregular bouts of high fever, prostration, weight loss, hepatosplenomegaly and anaemia may develop. As in subacute *P. falciparum* infections, leucopenia, thrombocytopenia, and high serum IgM levels can be found. Diagnosis can be difficult, as parasites are not continuously present.

([1]) The French literature calls this subacute form "paludisme viscéral évolutif".

P. ovale malaria

Clinical picture

P. ovale takes the place of *P. vivax* in regions in Africa where the Duffy blood group is absent. Clinically, *P. ovale* infections are very similar to *P. vivax* infection. The incubation can be relatively long, the early phase is frequently absent or unrecognised. Diagnosis may be difficult, since parasitemia may be very low for an impressive clinical picture, with shaking chills, high fever and prostration. Relapses present almost invariably as tertian fever and this may be an aid to diagnosis, together with haemolysis, thrombocytopenia and splenomegaly, in the absence of a positive thick film.

Case report

I (JVdE) happen to know P. ovale infection rather well, since I have had the disease myself. One year after leaving Zaire in 1985, I started having chills on a Monday night. Fever rose to 40°C. I made a few thick films, took some aspirin and went to bed where I started sweating heavily a few hours later. The next day, our specialised laboratory could not find any parasites in the thick films. On Wednesday night I had again shaking chills, which were a lot more severe. Fever rose to 40.5°C. Again I made thick films, quite sure that this was malaria. I took some aspirin, and sweating occurred a few hours later. The next morning, the same result came from the laboratory. I looked at the thick films myself, but was not able to find the slightest suspicion of a parasite. On Friday night, during a meeting at our institute, I started shaking again. Certain that it was malaria, given the undeniable periodicity, I took chloroquin after having taken several thick films. I had a terrible night with high fever, headache, myalgia and a kind of delirium. In the morning, the laboratory at first reported the thick films as negative but a concerted effort by two technicians and the chief parasitologist resulted in just one trophozoïte after one hour of searching!

P. malariae malaria

Classical presentation

If we do not take the renal complications into account, this is the least aggressive of all malaria species. However, the discussion about the anatomical origin of relapses, and the extreme life expectancy of this parasite make it the most enigmatic of all species.

Incubation is usually three weeks, and the early phase is generally very mild, without chills. Only fever and general malaise are reported. Anaemia is very uncommon.

The relapses are distinguishable from *P. vivax* or *P. ovale* in just one respect: in typical cases fever spikes are separated by two days, giving the quartan fever. Without treatment relapses die out after a few attacks, but can come back after years (up to 52 years after exposure!).

Renal involvement

Mostly in children, a glomerulonephritis can develop. Proof of a relationship between *P. malariae* and this membranoproliferative state comes from a geographic congruence, and from the demonstration of plasmodial antigen and antiplasmodial antibodies in glomerular deposits.

Albuminuria, the presence of casts in urine, and sometimes an overt nephrotic syndrome with oedema of face and ankles and ascites are clinical symptoms of the acute phase. This acute picture often evolves towards chronic glomerulonephritis and renal failure. Treatment with antimalarials is not effective in the affected individual, but malaria eradication campaigns were able to make this disease disappear in the past.

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CLINICAL FEATURES OF MALARIA IN PREGNANCY AND CHILDREN

H. M. Gilles

The first section of this paper will deal with the clinical features of malaria in pregnancy, while the second will cover the same topic in children.

Malaria in pregnancy

In areas of **unstable malaria** (and in non-immune pregnant mothers originating from non-endemic countries), pregnant women are susceptible to *all* the manifestations of severe malaria described in Chapter 7. Moreover, they have an increased risk of abortion, stillbirth, premature delivery and low birth weight of their infants (Wickramasuriya, 1935; Menon, 1972)[1, 2]. Malaria mortality rate in this group of pregnant women is 2-10 times higher than in non-pregnant women (Brabin, 1983 & 1991)[3, 4].

Falciparum malaria commonly induces uterine contractions and gives rise to premature labour. The frequency and intensity of contractions appear to be related to the height of the fever (Looaresuwan *et al.*, 1983)[5]. Foetal distress is common but often not diagnosed. The prognosis for the foetus is poor.

Only two of the common clinical features will be re-emphasised here, namely hypoglycaemia and pulmonary oedema.

Hypoglycaemia

Pregnant women are particularly prone to hypoglycaemia which may occur in otherwise uncomplicated disease. It is also commonly encountered on admission in severe disease or may result during the course of quinine therapy (White *et al.*, 1983)[6].

Hypoglycaemia may be asymptomatic or may present with sweating, abnormal behaviour, convulsions or a deteriorating level, or sudden loss of consciousness.

Acute Pulmonary Oedema

This complication is associated with a very high mortality. It may occur on admission; it may develop suddenly and unexpectedly at a time when other symptoms are subsiding and the parasitaemia has significantly dropped or even disappeared – or it may occur immediately after delivery (figure 1).

Fig 1. Acute pulmonary oedema in a pregnant woman (Copyright D. A. Warrell).

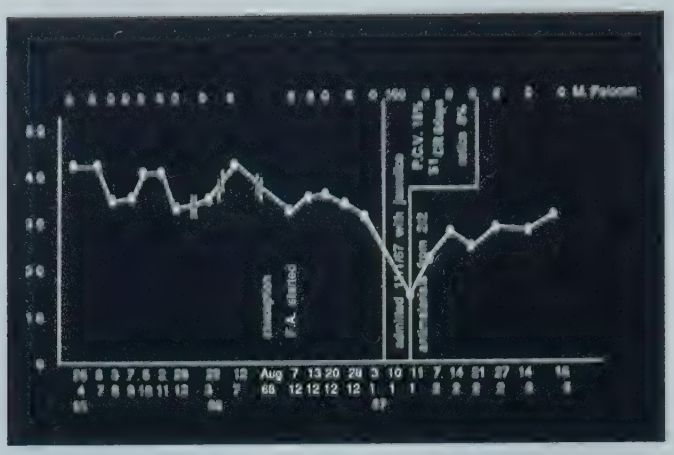

Fig. 2. Severe malarial anaemia in a primigravida, occuring in the 2nd trimester of her pregnancy.

In areas of **stable malaria** the classical manifestations of severe disease are rare in partially immune pregnant women. In these, and particularly in primigravidae, abortion, stillbirth, premature delivery and *low birth weight* of their new-born infants are the commonest findings (Wickramasuriya, 1935; Archibald 1956; Menon, 1972; Bray and Anderson 1979; McGregor *et al.,* 1983; Morgan 1994; Greenwood *et al.*, 1994; Brabin *et al.,* 1996)[1, 7, 2, 8, 9, 10, 11, 12]. Parasite rates and densities are greater in pregnant than non-pregnant women, especially in primiparae The other clinical consequence that is found is *anaemia* (figure 2), which bears little relation to the peripheral parasitaemia (Gilles *et al.*, 1969; Rougemont *et al.,* 1977; Brabin 1983; McGregor 1984; Steketee *et al.,* 1994; Matteelli *et al.,* 1994)[13, 14, 3, 15, 16, 17]. Once again, primiparae are much more likely to be affected.

Management

The management of hypoglycaemia, pulmonary oedema, anaemia and the other severe manifestations of malaria is similar to that described in Chapter on the "Treatment of malaria infection".

Monitoring of uterine contractions and foetal heart rate is imperative, while foetal or maternal distress may require early obstetric intervention, if labour has started.

Prevention

For *non-immune* pregnant mothers, chemoprophylaxis is advisable because malaria infection carries a greater risk of severe disease. In areas where resistance to chloroquine does not exist, or is predominantly RI, chloroquine with proguanil together with stringent personal protection measures (repellents, protective clothing, house screens, impregnated bed nets) often gives a good measure of protection. Breakthroughs can and do occur and must be diagnosed and treated promptly.

Mefloquine given from the second trimester onwards has proved safe and effective in areas where proguanil and chloroquine cannot be used (Steketee *et al.,* 1994; Nosten, 1994)[16, 18]. The increasing reports of side effects from this drug may limit its usefulness in the future.

For semi-immunes and particularly primigravidae, chemoprophylaxis has been shown to be effective in several trials in Africa, in reducing the two most important clinical consequences of malaria in this group – low birth weight of infants and anaemia. (Fleming *et al.*, 1986; Greenwood *et al.*, 1989; Greenwood *et al.*, 1994)[19, 20, 11]. Unfortunately, chloroquine which was the drug most frequently used is no longer effective in many areas. Mefloquine has been shown to be a good substitute, but it is expensive, it cannot be given in the first trimester and increasing reports of side effects are a cause for anxiety.

In order to overcome some of the problems encountered in the prevention of malaria in pregnancy, namely – increasing frequency and intensity of resistance to *P. falciparum*, contraindications of some of the antimalarials, poor compliance due to a variety of reasons – simplified regimes are undergoing clinical trials using sulphonamide-pyrimethamine combinations (where these are still effective, e.g. Africa) given at the first attendance (providing it is in the second trimester) and repeated once again at the beginning of the third trimester (WHO, 1994)[21].

Congenital Malaria

In endemic areas, vertical transmission of malaria across the placenta is common (Steketee *et al.,* 1994)[16], but parasitaemia often disappears within a few days after birth.

Symptomatic malaria – fever, anaemia, hepatosplenomegaly – are however rare (Cov-

ell, 1950; Logie and McGregor, 1970)[22, 23]. Passive immunity derived from the semi-immune mothers together with other factors e.g. high concentration of foetal haemoglobin, may be responsible (Edozien *et al.*, 1962; Pasvol *et al.*, 1980)[24, 25].

Malaria in Children

It is important to realise from the outset that malaria in indigenous children inhabiting highly endemic areas and thus continuously exposed to infection are, until the age of about 5 years highly susceptible to all the manifestations of *P. falciparum* malaria. Indeed, it is estimated that about 1,000,000 children aged between 1 and 5 years die every year from the disease. Because primary health services are poorly developed in many malaria endemic countries, it is very likely that most of these children die without reaching a health facility (Greenwood *et al.*, 1987)[26]. The children that survive this critical period, achieve, after several successive attacks, a relative tolerance of the infection – *semi-immune* – and in these, malaria is either asymptomatic despite a parasitaemia of 10% to 30%, or mild, with non-specific symptoms, such as mild fever, fretfulness, anorexia, slight sweating and slight anaemia. Hepatosplenomegaly is also common.

The incubation period of *P. falciparum* is 7-15 days; of *P. vivax* usually 10-20 days; of *P. ovale* 11-16 days and of *P. malariae* 30-40 days or longer.

Clinical features

a) ***P. Falciparum malaria***

The non-immune child with *uncomplicated* malaria often appears listless, restless or drowsy, refuses food and if old enough, may complain of headache or nausea. Pallor of the skin, nails and mucous membranes may occur. Thirst may be marked as the temperature rises; breast-fed infants make frequent attempts to suck the breast, but this is soon abandoned, possibly due to nausea. A clear cut cold stage with rigor, hot stage and sweating stage are uncommon. Vomiting is often marked and the vomitus, bile-stained; while the stools may

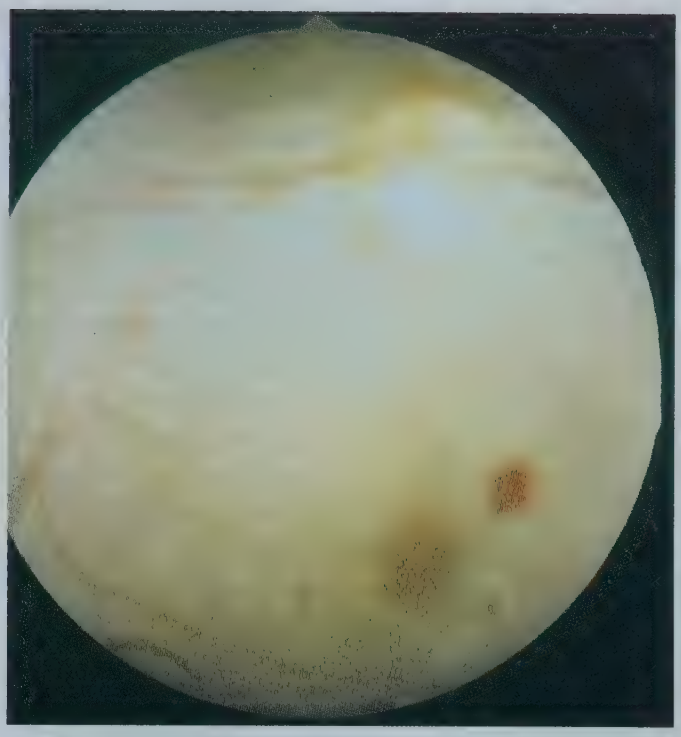

Fig. 3. Retinal haemorrhages in malaria (Copyright D A Warrell).

be loose and dark green, but no blood or pus cells are seen. Infants may seem in abdominal distress while older children complain of pain over the liver or spleen. Constipation is not uncommon. The temperature is often high (40°C), continuous and irregular and the child is flushed and sweaty. Even when the temperature is only moderately high, febrile convulsions can often occur. Unless consciousness returns within about 30 minutes, cerebral malaria must be suspected. The liver is often enlarged and tender, followed by enlargement of the spleen. Opthalmological examination of the fundi should be carried out because the presence of retinal haemorrhages is an indication of impending severe disease in some areas of the world (figure 3).

The diagnosis of uncomplicated malaria in semi-immune children is more complex because so many of them can have an *asymptomatic* parasitaemia ranging from 2%-30%, hepatosplenomaly and their symptoms may be due to a variety of other illnesses; the parasitaemia being incidental rather than causal. As reasonable a set of criteria as any for the diagnosis of uncomplicated malaria in these children is (1) fever, for which no other cause can be found on physical examination, (2) a parasitaemia of 10,000 parasites per ul if mi-

croscopic examination is available, (3) response of fever and other symptoms within 48-72 hours, (4) diminution of parasitaemia unless RII resistance is suspected (Gilles & Warrell, 1993)[27].

Since microscopical facilities are rarely available in primary health centres, other definitions have been sought. The most recent clinical algorithm has identified three signs – temperature 37.7°C or higher; splenomegaly or pallor of the nailbeds. In Malawi, this combination was 85% sensitive in identifying parasitaemic children and 41% specific (Redd *et al.*, 1996)[28]. The recently introduced *Integrated Management of the child* initiative by WHO is a very welcome advance in this context.

A delay in diagnosis and treatment in *non-immune* children as short as *two to three days* usually results in the development of the various syndromes associated with severe *falciparum malaria* (see Chapter 7). Some of these, namely *renal failure*; *pulmonary oedema* and *spontaneous bleeding* (DIC), are rare in children and have already been described in Chapter 7. The commonest and most important manifestations of *severe malaria* in children are: (1) *cerebral malaria* (2) *anaemia* (3) *metabolic acidosis* (4) *hypoglycaemia* (5) *hyperpyrexia* (6) *hyperparasitaemia* (7) *circulatory collapse* (8) *haemoglobinuria*. In this context, it is important to remember that multiorgan failure i.e. *the presence of more than one of the above manifestations occurring in the same patient is a common occurrence in severe malaria*.

1) Cerebral malaria

Cerebral malaria *usually but not always* presents with fever (37.5-41°C), and a history of failure to eat or drink, vomiting, cough, impaired consciousness and moderate dehydration. Diarrhoea is unusual. Hepatosplenomegaly is usual. Convulsions are common and may occur at any age and any temperature. The depth of coma is variable and should be assessed using the Blantyre scale and regularly monitored throughout the course of the illness. In some children, the breathing is laboured and noisy; deep breathing with a clear chest clinically and/or radiologically suggests acidosis (see later). Retinal abnormalities including haemorrhages, vessel obstructions, macular or extramacular oedema and papilloedema occur. Extra macular retinal oedema was associated with a 2.9 fold increase in the relative risk of dying while the relative risk of death in patients with papilloedema was 6.7 times that in patients without papilloedema (Llewallen *et al.*, 1996)[29]. Jaundice and spontaneous bleeding are rare (Molyneux *et al.*, 1989)[30].

Neurological abnormalities are very variable and include opisthotonos (figure 4), decerebrate or decorticate rigidity, hypotonia, abnormal plantar reflexes, absent abdominal, corneal and vestibulo-ocular reflexes, conjugate deviation of the eyes and grinding of the teeth (Bruxism) (Molyneux *et al.*, 1989; WHO, 1997)[31, 32]. CSF opening pressure is raised (Newton *et al.*, 1991)[33].

Plasma urea and creatinine concentrations are often elevated on admission, but revert to normal following rehydration. Hypoglycaemia and acidaemia (particularly hyperlactataemia) are often associated with cerebral malaria (White, 1986; Taylor *et al.*, 1993; Marsh *et al.*, 1995; WHO, 1997)[34, 35, 36, 32].

Most surviving children with cerebral

Fig. 4. Opisthotonos in a child with cerebral malaria (Copyright M E Molyneux).

malaria make a full neurological recovery, but about 11% develop neurological sequelae. The most reported sequelae include ataxia, hemiplegia, speech disorders, blindness, behavioural disturbances, hypotonia, general spacticity, tremors, visual and auditory hallucinations (Brewster *et al.*, 1990; WHO, 1997)[37, 32].

Fortunately, most children with sequelae show considerable improvement and sometimes even complete recovery after the passage of time (Schmutzhard *et al.*, 1984; WHO, 1997)[38, 32].

2) Anaemia

Anaemia is an important manifestation of severe malaria. It is most commonly seen in children under 2 years of age. It may occur alone or in combination with other manifestations of severe malaria e.g. cerebral malaria; metabolic acidosis. Breathing difficulties in an anaemic child are more likely to be due to metabolic acidosis than cardiac failure (WHO, 1997)[32].

The rate of development and the degree of anaemia depend on the severity and frequency of parasitaemia. In some children, repeated untreated or inadequately treated attacks of otherwise uncomplicated malaria will result in a progressive normochromic anaemia in which dyserythropoietic changes in the bone marrow are prominent. Parasitaemia is often scanty, and a presumptive diagnosis of a malarial aetiology is based on finding numerous pigmented monocytes in the peripheral blood.

In other children, severe anaemia may develop rapidly in association with hyperparasitaemia. In these cases, acute haemolysis of parasitized red cells is mainly responsible. Severe anaemia is however multifactorial and the haematological picture may be variable.

3) Metabolic acidosis

Acidaemia (defined as an arterial pH less than 7.25) or acidosis (plasma bicarbonate concentration less than 15 mmol/l), is an important manifestation of severe malaria. It can occur independently, or in association with other manifestations of severe malaria, particularly cerebral malaria, anaemia, hypoglycaemia and hypovolaemia (Taylor *et al.*, 1993; Krishna *et al.*, 1994; Marsh *et al.*, 1995)[35, 39, 36]. The major contribution to acidosis is hyperlactataemia(5 mmol/l; normal range up to 2 mmol/l). Lactic acidosis should be suspected if the anion gap ($Na^+ - (Cl^- + HCO_3^-)$ mEq/l exceeds 10-12 mEq/l).

Respiratory distress manifested by deep breathing (increased inspiratory and expiratory excursion of the chest) is a reliable clinical indicator of metabolic acidosis. It is important to note in this context that the overlap between severe malaria presenting with respiratory distress and acute respiratory infections (ARI) is considerable. It is extremely difficult on clinical grounds to distinguish between severe malaria presenting with cough, breathing difficulties and a raised respiratory rate, and pneumonia (O'Dempsey *et al.*, 1993)[40].

In situations where neither x-ray facilities nor microscopic examination are available, or when in doubt, it is prudent to treat children presenting with fever and respiratory symptoms, for both malaria and ARI (WHO, 1997)[32].

4) Hypoglycaemia

Hypoglycaemia commonly occurs in children under 3 years. It is a frequent presenting symptom in malaria, as well as other childhood illnesses. It is associated with convulsions, profound coma, hyperparasitaemia, lactic acidosis and high levels of circulating tumour necrosis factor (WHO, 1997)[32].

In contrast to adults, it rarely occurs as a result of quinine therapy. It is easily overlooked clinically because its features may be similar to those of cerebral malaria. It is imperative therefore to measure on admission whole blood glucose of all children suffering from severe malaria (stix method and/or biochemically).

5) Hyperpyrexia

High fever is common in children suffering from severe malaria. Although convulsions may occur at any level of body temperature, they more commonly occur at temperatures above 38.5°C (Familusi & Sinnette, 1971)[41]. Hyperpyrexia can result in coma.

6) Hyperparasitaemia

The interpretation of hyperparasitaemia varies in different areas, in unstable areas, a peripheral parasitaemia in non-immune children of 4% is indicative of severe malar-

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ia (WHO, 1997)[32]. In areas of stable malaria, threshold levels of 20% or greater should be considered to indicate severe malaria (WHO, 1997)[32], although local experience, if available, may prove otherwise.

7) Circulatory collapse (see also Chapter on the "Clinical aspects of malaria")

The diagnosis of shock in children is based on a combination of hypotension (BP 50 min/Hg. or less), weak or absent peripheral pulses and coolness of the mid to proximal limbs.

8) Haemoglobinuria (see also Chapter on the "Clinical aspects of malaria")

Dark red or black urine occurs in children usually associated with hyperparasitaemia in the absence of G6.P.D. deficiency.

Diagnosis

The differential diagnosis of severe malaria in children will have to take into consideration the common childhood diseases, especially if the child has not been immunised against them e.g. diphtheria and tetanus. A bulging fontanelle or neck stiffness would suggest meningitis; although its absence does not exclude it. Sepsis in any limb or organ, should be looked for. Tonsillitis and chronic otitis media should be excluded.

As has already been pointed out, two of the most important differential diagnoses are *acute respiratory infection* (ARI) and pneumonia. Enlarged lymph nodes could be a pointer to other alternative diagnoses – e.g. trypanosomiasis, tuberculosis and other numerous possibilities.

A rash would suggest typhus, typhoid, an arbovirus infection, relapsing fever, chickenpox, measles, or menigococaemia.

The differences between severe malaria in adults and children are given in Table 1.

Management

The management of severe malaria in children is basically similar to that described for adults; a summary of which is given in Table 2. A few particular measures will be re-emphasised here. The most important maxim is frequent monitoring and reassessment; initially at hourly or 2-hourly intervals, if possible.

Convulsions

It is important to note whether the child has received diazepam or paraldehyde before admission to hospital and when, since this is a frequent occurrence, and another injection is inadvisable.

Diazepam should be given intravenously by slow injection at an initial dose of 0.3

Sign or Symptom	Adults	Children
Cough	Uncommon early symptom	Common early symptom
Convulsions	Common	Very common
Duration of illness	5-7 days	1-2 days
Resolution of coma	2-4 days	1-2 days
Neurological sequelae	Uncommon	10% (approx)
Jaundice	Common	Uncommon
Pretreatment hypoglycaemia	Uncommon	Common
Pulmonary oedema	Common	Rare
Renal failure	Common	Rare
CSF opening pressure	Usually normal	Often raised
Bleeding/clotting disturbances	Up to 10%	Rare
Abnormality of brain stem reflexes (e.g. oculovestibular, oculocervical)	Rare	More common

Table 1. Differences between severe malaria in adults and in children. Derived from studies in South-East Asian adults and African children (Warrell *et al.*, 1989; Molyneux *et al.*, 1989: WHO, 1997)[30, 32].

Manifestation/complication	Immediate management[a]
1. Coma (cerebral malaria)	Maintain airway; nurse on side; exclude other treatable causes of coma (eg hypoglycaemia, bacterial meningoencephalitis). Give prophylactic anticonvulsant (10 mg of phenobarbitol sodium per kg of body weight intramuscularly). Avoid harmful adjuvant treatments such as corticosteroids, heparin and epinephrine (adrenaline)
2. Convulsions	(Prevent with intramuscular phenobarbitol sodium, see above). Maintain airway; treat with diazepam given intravenously or per rectum (0.15 mg/kg to a maximum of 10 mg) or intramuscular paraldehyde injection (0.1 ml/kg from a glass syringe)
3. Severe anaemia	Transfuse fresh whole blood or packed cells
4. Acute renal failure	Exclude dehydration; maintain strict fluid balance; carry out peritoneal dialysis (or haemodialysis if available)
5. Hypoglycaemia	Measure blood glucose, give 50% glucose injection 50 ml (1ml/kg for children) followed by 5% or 10% glucose infusion
6. Metabolic acidosis	Exclude or treat hypoglycaemia, anaemia, hypovolaemia and Gram-negative septicaemia. Give oxygen. Correct arterial pH to 7.2 or above. Blood transfusion. Crystalloid infusion
7. Acute pulmonary oedema	Prevent by avoiding excessive rehydration. Prop patient up; give oxygen. If the pulmonary oedema is due to overhydration, stop intravenous fluids, give diuretic (frusemide 40 mg intravenously) and withdraw 250 ml of blood by venesection into a donor bag
8. Shock, algid malaria	Suspect Gram-negative septicaemia; take blood samples for culture. Give parenteral antimicrobials; correct haemodynamic disturbances
9. Spontaneous bleeding and coagulopathy	Transfuse fresh whole blood or clotting factors; give vitamin K injection
10 Hyperpyrexia	Use tepid sponging and fanning; give antipyretic (paracetamol 15 mg/kg of body weight)
11. Hyperparasitaemia	Give initial dose of parenteral antimalarial therapy. If parasitaemia in a severely ill patient exceeds 10%, carry out exchange or partial exchange transfusion
12. Malarial haemoglobinuria	Continue antimalarial treatment; transfuse fresh blood to maintain haemocrit above 20%; give frusemide 20 mg intravenously
13. Aspiration pneumonia	Give parenteral antimicrobials; change position of patient; give physiotherapy; give oxygen

[a] In all cases, infusion or injection of an appropriate antimalarial drug should be started immediately.

Table 2. Summary of the management of severe and complicated *Falciparum malaria* (Gilles, 1991)[42].

mg/kg (rate not to exceed 2 mg/min) or interectally at a dose of 0.5 mg/kg.

Paraldehyde is safe and effective and can be given to children who have already received diazepam. The dose is 0.2 ml/kg by deep intramuscular injection, or 0.4 ml/kg intrarectally.

Hypoglycaemia

This is treated with 25% or 50% dextrose (0.5 g m/kg) given intravenously over several minutes. Glucose solution can be given by nasogastric tube if intravenous infusion is not possible.

Metabolic acidosis

Correct any reversible cause of acidosis; particularly dehydration and severe anaemia. If the haemoglobin is < 5 g/dl and the child has respiratory distress, blood transfusion 20 ml/kg given over 4-6 hours is given. If the haemoglobin is >5 g/dl with respiratory distress, give normal saline in aliquots of 10 ml/kg, the speed of infusion being based on clinical judgement (WHO, 1997)[32]. If any doubt exists as to whether ARI may be the cause of respiratory distress, give parenteral antibiotic therapy in addition to the above management.

Severe anaemia

The decision of when to transfuse must be based on careful clinical judgement and particular needs of individuals, rather than on a laboratory-based formula.

Chemotherapy

The Chemotherapy of severe malaria in children is given in the chapter on "Treatment of malaria infections". Areas of the world where chloroquine resistance has not been reported are so few and the resistance situation so dynamic that *quinine is the drug of choice*.

In circumstances where one can be *certain* that *P. falciparum* parasites are fully sensitive to chloroquine, then this drug is preferable to quinine – it acts faster and does not produce hypoglycaemia. It must however be given in the correct dosage, correct route, correct rate of infusion and frequency.

If for some reason quinine or quinidine is not available, then it is justified to use parenteral chloroquine, even if resistance is known to occur, in the hope that the majority of cases would only be RI or RII resistant.

In areas where *multidrug resistance* occurs, artemisinin and its analogues are the drugs of choice given by intramuscular or intravenous injection, or as suppositories (White, 1996)[43].

Prognosis

The major indicators of a poor prognosis in children with severe malaria are listed below (Grau *et al.*, 1989; WHO, 1997, White, 1996)[44, 32, 43].

A. **Clinical Indicators**
   - Depth of coma
   - Witnessed convulsions
   - Age < 3 years
   - Papilloedema and/or retinal oedema
   - Respiratory distress (deep inspiratory and expiratory breathing)
   - Absent corneal reflexes

B. **Laboratory Indicators**
   - Parasite density > 1 million/ul
   - Peripheral schizontaemia
   - Peripheral leucocytosis (> 12,000/µl)
   - Large size of ring-stage parasites in peripheral blood
   - Intraleucocytic malaria pigment (Nguyen *et al.*, 1995)[45]
   - Hypoglycaemia (< 2.2 mmol/l-< 40 mg/dl)
   - Metabolic acidosis (pH < 7.3: base-excess < –12)
   - Increased plasma lactate concentrations (> 6 mmol/l)
   - Increased CSF lactate concentrations (> 6 mmol/l)
   - Severe anaemia (Hb < 5.0 g/dl or PCV < 20%)
   - Increased plasma concentrations of tumour necrosis factor (TNF) (Kwiatkowski *et al.*, 1993)[46]
   - Raised plasma creatinine (Allen *et al.*, 1996)[49]
   - Decreased plasma bicarbonate (Allen *et al.*, 1996)[49]

It must be appreciated however, that regional differences in presentation, prognostic factors and mortality, occur. Thus, whereas cerebral malaria seems to be the commonest presentation in West Africa; anaemia is the commonest presentation in Papua New Guinea (Waller *et al.*, 1995; Modiano *et al.*,

1995; Allen *et al.*, 1996)[47, 48, 49]. Large size ring-stage parasites in the peripheral blood are sometimes seen in asymptomatic children in stable areas of West Africa.

b) *Vivax and Ovale malaria*

Ovale malaria is clinically milder than vivax, otherwise, the clinical features are similar. Some strains of *P. Vivax*, especially those from temperate regions, have a long incubation period, up to 8-14 months.

Prodromal symptoms are non-specific and include restlessness, drowsiness, anorexia, headache and nausea. Fever is at first irregular, remittent, or quotidian, later becoming tertian if the child is not treated early. It occurs by day and usually develops in the afternoon. Febrile convulsions are not uncommon but cerebral malaria does not occur. The classical febrile paroxysm consisting of the cold stage (15 m-60 m); the hot stage (2 hours or more) and the sweating stage are uncommon in infants and children. Hepatosplenomegaly is common and a moderate anaemia may be found. Parasitaemia may be less than 2% of erythrocytes infected; all stages of the asexual cycle are usually found in the peripheral blood, with gametocytes appearing after a week of the clinical course.

If a blood schizonticide only is given, relapses occur after a period of quiescence, weeks or months after the initial attack.

The *treatment* of vivax and ovale malaria is given in Chapters on the "Treatment of malaria infections".

Resistance of *P. Vivax* to chloroquine has been reported (Papua New Guinea, Irian Jaya) but is at present extremely rare. For practical purposes, chloroquine remains the drug of choice for the treatment of *vivax* and *ovale* malaria. Radical cure can only occur if primaquine is given in addition to chloroquine. Some strains e.g. Chesson strain of *P. vivax* require the longer course of primaquine recommended (21 days). It is only advocated if the child no longer continues to live in an endemic area. If he/she continues to live in an endemic area and is therefore at continual risk of reinfection, treatment of the overt attack only with chloroquine is advocated.

If the child has G6PD deficiency a weekly dose of primaquine of 7.5mg/kg for 8 weeks, also produces a radical cure and diminishes the risk of a drug-induced haemolysis.

c) *Quartan malaria (Malariae malaria)*

The clinical picture is basically similar to that of *vivax* malaria, except that the periodicity of the fever, when established, is quartan (every 72 hours) instead of tertian (every 48 hours).

The most important clinical manifestation is the development of the nephrotic syndrome, often referred to as *quartan malarial nephropathy* (QMN). Despite numerous reports on the association between *P. malariae* and the nephrotic syndrome (figure 5), a notable tone of incredulity still existed among physicians, both in non-endemic and endemic malaria areas. Thus, Luder (1958)[50] concluded that the association was still unproven, while Trowell (1960)[51] suggested that the time was ripe for the reassessment of the whole problem. The association was first confirmed statistically in Nigeria (figure 6) by Gilles and Hendrickse (1963)[52] and later in East Africa (Kibukamusoke *et al.*, 1967; Kibukamusoke, 1973)[53, 54]. Meanwhile, the decline in the prevalence of the nephrotic syndrome which followed malaria eradication in Guyana lent further support for the causal relationship (Giglioli, 1962)[55]. It was postulated that the most likely mechanism was an immune complex nephropathy (Gilles and Hendrickse, 1963)[52]. Subsequent immunological studies confirmed the hypothesis that glomerular damage was due to immune complex deposition (Ward and Kibukamu-

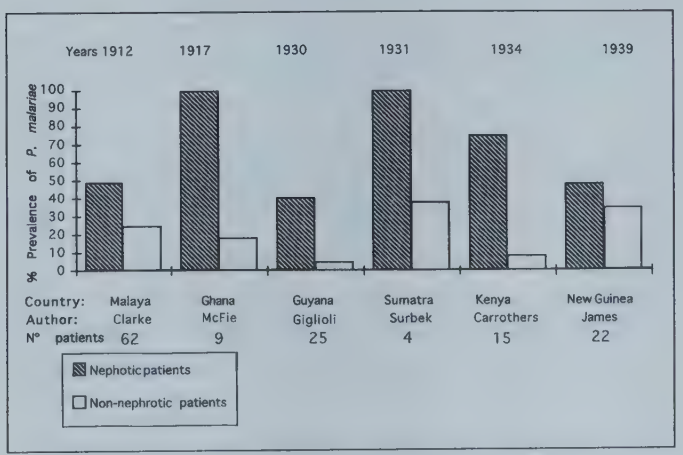

Fig. 5. World-wide reports on the association between *P. malariae* and the nephrotic syndrome.

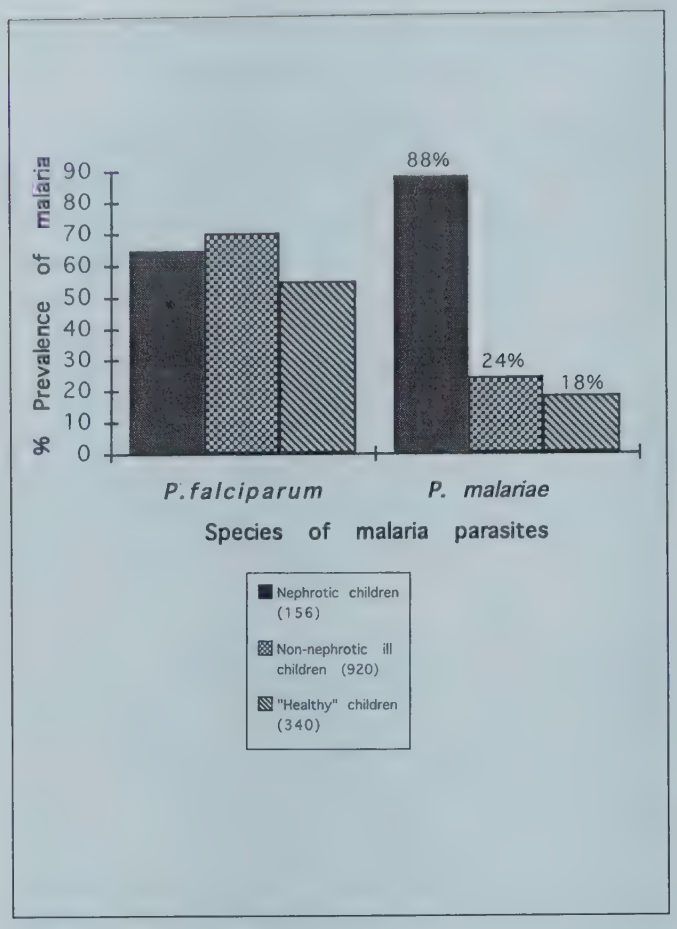

Fig. 6. Prevalence of *P. malariae* in nephrotic and non-nephrotic Nigerian children (aged 2-10 years).
Overall P. malariae infection rate:
(1) Nephrotic (113) – non-nephrotic ill children (920)
$X^2 = 138.3$; n=1; P. < 0.001
(2) Nephrotic (113) - unselected "healthy" village children (340)
$X^2 = 177.1$; n=1; P. < 0.001
No statistical difference in *P. falciparum* infection rates in the three groups was found.

Fig. 7. Nigerian child with quartan malaria nephropathy.

soke, 1969; Hendrickse *et al.*, 1972)[56, 57] and the presence of *P. malariae* antigen in the glomeruli was demonstrated. Renal biopsy studies suggested that there may be a morphologically specific lesion.

Children between 4-8 years are most commonly affected and they usually present with severe generalised oedema; persistent heavy proteinuria; severe proteininaemia and ascites (figure 7); azotaemia or hypertension are very uncommon.

Symptomatic and supportive treatment consists of diuretics and high protein diet. Antimalarial, steroid and immunosuppressive therapy of QMN have given disappointing results (Gilles and Hendrickse, 1963; Adeniyi *et al.*, 1970; Wing *et al.*, 1972)[52, 58, 59].

The treatment of *uncomplicated* quartan malaria is given in the chapter on the "Treatment of malaria infections".

Diagnosis

The diagnosis of malaria in children can be conveniently divided into (i) clinical, (ii) parasitological (iii) immunological and (iv) molecular.

From the *clinical standpoint*, the most important factor is to have a *high index of suspicion* that malaria is a likely diagnosis. It must always be appreciated that malaria can clinically mimic many other diseases. The commonest misdiagnoses in children are ARI, pneumonia and meningitis.

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DIAGNOSIS OF MALARIA INFECTION

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Introduction

The correct and timely diagnosis of malaria is of critical importance since the life-threatening severe form may quickly develop, possibly leading to death despite intensive care. The technical capability to confirm malaria infection by laboratory means is of utmost importance in several settings, not only in clinical medicine in endemic and non-endemic countries, but also for epidemiological and research purposes.

This chapter will describe the different laboratory tools for confirmation of infection. Given the practical purpose of this handbook, which addresses health workers in the field, we will mainly concentrate on common affordable technology, even though brief notes on modern sophisticated diagnostic tools will also be offered to the reader.

In the next chapter we will focus on the interpretation of the results in different settings, combining them with symptoms and signs.

Clinical presumptive diagnosis

The appearance of clinical symptoms is usually the reason that urges the ill patient in industrialized non endemic countries to turn to the physician. Clinicians in western countries need to be well aware of the possibility of imported malaria cases. Diagnosis in these cases may be made difficult by the possibility that the exposure to the infective bite may date back to a long time before clinical symptoms appear, especially in the case of *P. vivax* or *P. malariae* infections. The accurate geographical history (*unde venis?)* should be a routine in every history taking of every patient even in the malaria-free world. Finally, it is to be stressed that *P. falciparum* malaria in non immune subjects coming from non endemic western countries may be extremely severe, with high case-fatality rates if correct diagnosis and treatment is not carried out promptly.

In malaria endemic developing countries, on the contrary, economic and logistic reasons force entire populations to rely esclusively on self diagnosis and treatment, even in the case of moderate fever. It is to be noted, in fact, that semi-immunity usually leads to mild to moderate clinical disease in adults living in endemic countries. Unfortunately, the clinical symptoms of mild to moderate malaria in these cases are extremely aspecific (fever, headache, myalgias, etc.), mimicking a large series of other clinical conditions. On the contrary, clinical symptoms may be severe and disease may be lethal in children and in selected adult patients even in endemic areas.

The extremely wide spectrum of haema-

Presenting symptom/sign	Notes	Differential diagnosis
Fever	Often irregular or continuous at onset	Influenza, dengue, brucellosis, sandfly fever, acute respiratory infections, viral hemorragic fevers, tuberculosis, enteric fever, trypanosomiasis, visceral leishmaniasis, rickettsial fever, relapsing fever
Coma	Usually (not always!) high parasitaemia is present	Meningitis, encephalitis, typhoid fever, erebrovascular accidents, trauma, alcoholism, hypoglicemia
Jaundice	More common in severe malaria for which it is a defining criteria	Infectious hepatitis, leptospirosis, disease of the biliary tract
Hepatomegaly	Usually tender in the acute phase	Amoebic liver abscess, beri-beri, brucellosis, leptospirosis, relapsing fever, viral hepatitis, thalassaemia
Splenomegaly	Common in all types of malaria and usually tender in the acute phase	Brucellosis, relapsing fever, trypanosomiasis, typhoid fever, leptospirosis, thalassaemia
Renal failure		G6PD deficiency, sickle cell disease, snake venoms, leptospirosis, traditional herbal remedies, heat stroke
Gastrointestinal symptoms	All kinds of gastrointestinal symptoms (ranging from vomiting to choleraic diarrhea)	All types of dysentery, cholera, gall bladder disease, acute pancreatitis, acute appendicitis

Table 1. Differential diagnosis of the presenting clinical symptoms/signs in *falciparum malaria* (adapted from Gilles, 1988)[4].

tological signs and symptoms occurring during malaria infection is covered in the chapter on *Clinical features of malaria infection in man* and exceeds the purpose of this chapter. A brief and non exhaustive list of possible differential diagnosis of *falciparum* malaria presenting symptoms and signs is nevertheless indicated in Table 1.

Whenever possible, it is recommended to perform at least a simple panel of haematological blood examinations (white blood cell count, red blood cell count, haematocrit, haemoglobin level, platelet count, etc.), the predictive positive value of some of which may be very high in some endemic malaria areas. Polyfactorial haemolitic *anaemia* is very common in falciparum malaria and it is caused by direct lysis of red blood cells, inappropriate bone marrow response and autoimmune disorders (World Health Organization, 1990)[1]. Platelet levels are very seldom normal in malaria infections caused by all species and often fall below 50.000/µl in *falciparum* malaria. The predictive positive value for *falciparum* malaria given the *triade* fever, history of recent stay in tropical areas and *thrombocytopenia* below 100.000/l has been found to be as high as 97% in migrants to some non endemic areas (Castelli *et al.*, 1995)[2]. No specific diagnostic indications are given by the white blood cell count during malaria attack since leukocytosis is usually absent in the absence of concomitant bacterial infections (Wery, 1991)[3].

Wherever the laboratory confirmation of malaria is not available in highly endemic areas, the experienced clinician may diagnose malaria with sufficient predictive positive value for positive blood slide (Genton *et al.*, 1994)[5], even though contrasting reports exist indicating that the predictive positive value of clinical signs to diagnose malaria

may be poor during low transmission seasons (Sowunmi and Akindele, 1993)[6].

Laboratory diagnosis

Once malaria is suspected on clinical grounds, it is mandatory to obtain the laboratory confirmation of the presence of malaria parasites in the patient's organism, whenever possible. The diagnosis of malaria may in fact be pursued by the direct demonstration of the parasite whole cell or of parasite's nucleic acid or products in the blood (*direct diagnosis*) or by the demonstration of the patient's immune response to the infection (*indirect diagnosis or immunodiagnosis*).

Direct diagnosis of malaria infection has been, and still is, the cornerstone of malaria diagnosis since 1880, when the French Army surgeon Alphonse Laveran first observed and described malaria parasites in human blood in Algeria.

The direct demonstration of the whole parasitic cell may be accomplished by several methods, from the old and simple (but still golden standard!) direct microscopic observation of stained blood specimen to the more recent and sophisticated concentration and staining techniques (Quantitative Buffy Coat®, acridine orange method). Finally, the detection of the presence of the genome of the parasite is now possible by the Polymerase Chain Reaction (PCR) technique. New and sensitive immunological methods to detect *P. falciparum* antigens have yielded promising results for the individual diagnosis of malaria infection.

The evidence of seric specific antibodies is of little, if any, diagnostic value in endemic malaria areas but may provide important information in epidemiological studies and, in some selected setting, in blood donor screening. New and sensitive immunological methods to detect *P. falciparum* antigens have yielded promising results for the individual diagnosis of malaria infection.

Direct parasitological diagnosis

This chapter will focus particularly on the simple direct microscopic observation of blood specimen, but informations on the newer diagnostic tools (QBC®, PCR) will also be given to facilitate the reader's comprehension of the new developments in malaria operational research which may hopefully bear fruit at the field level in the new future.

a) Light microscopic observation

At the end of the last century, it was recommended that the direct microscopic observation of malaria parasites be done in fresh unstained blood specimens flattened between a slide and a cover slip to appreciate the mobility of the parasite. It was then soon recognized by Romanowsky (1890) that the fixation and staining of blood specimens permitted a more accurate definition of the different stages and species, even though mobility was lost. The current standard methodology of Giemsa stained blood thick smears for the diagnosis of malaria dates back to 1903 (Ross, 1903)[7].

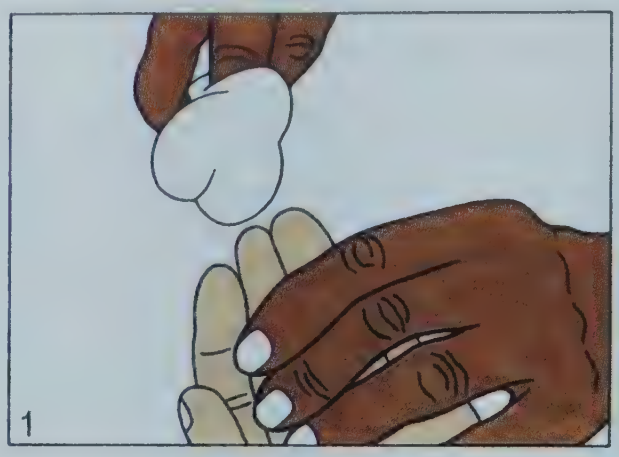
Though simple in principle, the direct microscopic observation of stained blood specimens requires specific instrumentation and reagents (microscope, microscopic slides, pricking needle, staining reagents, water, electric or solar light, etc. – see Annex 1) and a trained professional to obtain to a correct diagnosis. Information on basic microscopy, the basic staining procedures and on the most common mistakes are given in this subchapter together with the morphological clues to make an adequate *qualitative* (stage and species diagnosis) and *quantitative* (parasitaemia) diagnosis of malaria infection at the district level.

Excellent reviews on microscopic malaria diagnosis, to which continuous reference will be made in the text, are those from Shute (1988)[8], Lopez Antunano (1990)[9], the World Health Organization (1991)[10] and Gilles (1993)[11].

Blood drawing technique

Since parasite concentration is fairly constant in internal and peripheral blood, it is routine to draw the blood by pricking a finger (or the heel in young infants) with a sharp sterile needle. The best site to prick is the lateral side of the third phalanx of the second or third finger of the left

After details about the patient have been recorded in the appropriate form or register, the blood films are made as follows:



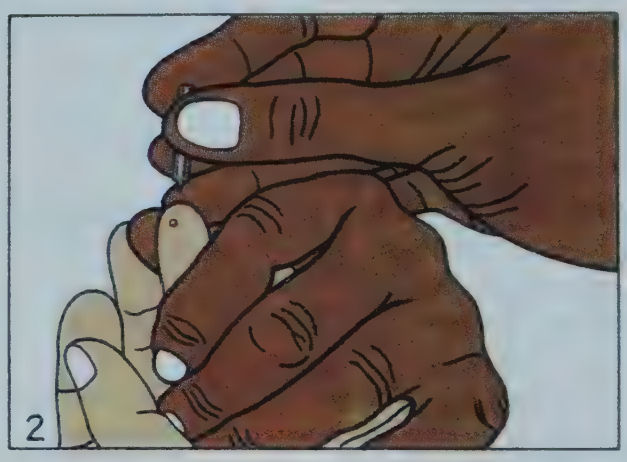
1. Holding the patient's left hand, palm upwards, select the third finger from the thumb. (The big toe can be used with infants. The thumb should never be used for adults or children).

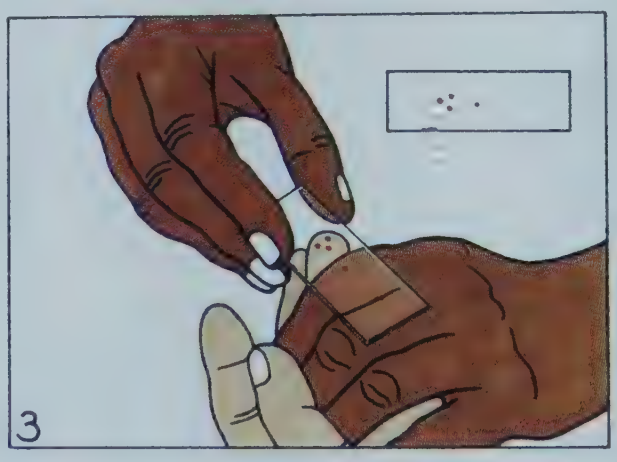
   Clean the finger with a piece of cotton wool lightly soaked in alcohol, using firm strokes to remove dirt and grease from the ball of the finger.

   Dry the finger with a clean cotton cloth, using firm strokes to stimulate blood circulation.

2. Puncture the ball of the finger with a sterile lancet, using a quick rolling action.

   Apply gentle pressure to the finger to express the first drop of blood and wipe it away with a dry piece of cotton wool. Make sure that no strands of cotton remain on the finger to be later mixed with the blood.





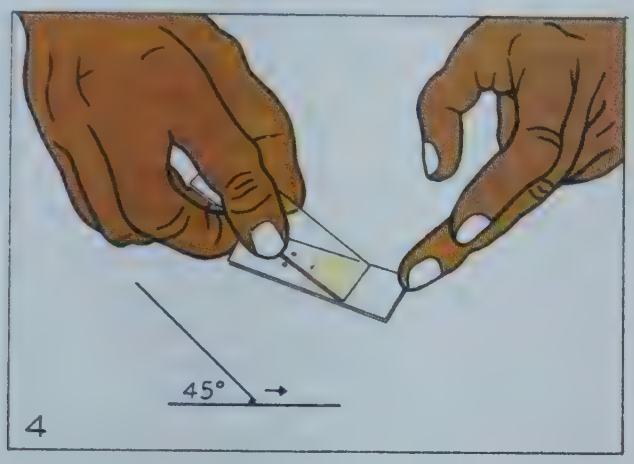
3. Working quickly and handling clean slides only by the edges, collect the blood as follows.

   Apply gentle pressure to the finger and collect a single small drop of blood, about this size ●, on the middle of the slide. This is for the thin film.

   Apply further pressure to express more blood and collect two or three larger drops, about this size ●, on the slide, about 1 cm from the drop intended for the thin film (see illustration).

   Wipe the remaining blood away from the finger with a piece of cotton wool.

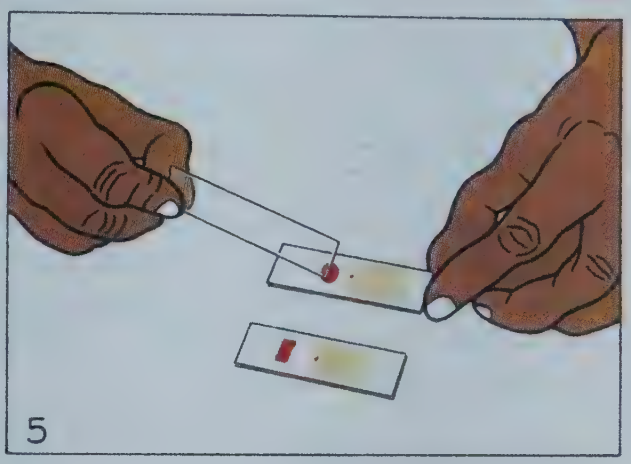
Fig. 1. Preparation of thick and thin blood films on the same slide (reproduced by permission of WHO, from: *Ba*

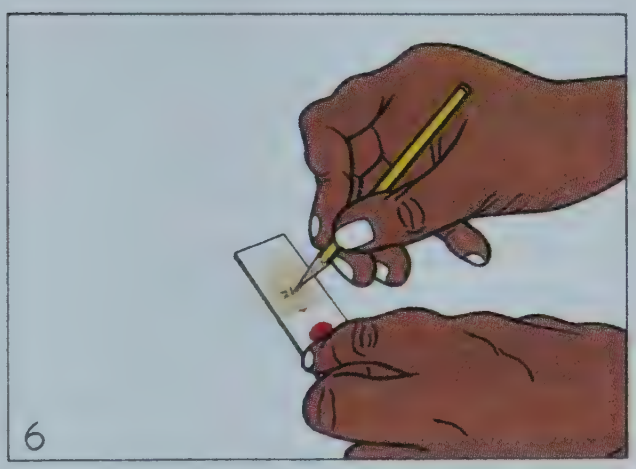


4. *Thin film.* Using a second clean slide as a "spreader" and, with the slide with the blood drops resting on a flat, firm surface, touch the small drop with the spreader and allow the blood to run along its edge. Firmly push the spreader along the slide, keeping the spreader at an angle of 45°. Make sure that the spreader is in even contact with the surface of the slide all the time the blood is being spread.

5. *Thick film.* Always handle slides by the edges or by a corner to make the thick film as follows.

   Using the corner of the spreader, quickly join the drops of blood and spread them to make an even, thick film. The blood should not be excessively stirred but can be spread in circular or rectangular form with 3 to 6 movements. The circular thick film should be about 1 cm ($\frac{1}{3}$ inch) in diameter.





6. Label the dry thin film with a soft lead pencil by writing across the thicker portion of the film the patient's name or number and the date. Do not use a ball-point pen for labelling the slide. Allow the thick film to dry with the slide in a flat, level position, protected from flies, dust and extreme heat.

7. Wrap the dry slide in the patient's record form and dispatch it to the laboratory as soon as possible.

8. The second slide used for spreading the blood films may now be used for the next patient and another clean slide from the pack will be used as a spreader.

Example of well made and correctly labelled thick and thin films

a *Microscopy. Part I. Learner's guide.* Geneva, World Health Organization, 1991).

hand (unless the patient is left-handed). After accurate cleaning with spirit-moisted cotton, pricking is carried out by the health worker and the first drop of blood must be removed with cotton. The following drops of blood, obtained by gentle squeezing of the finger by the health worker, are then collected on a glass slide that is lowered to touch the top of the drop (see figure 1). It is to be stressed that the glass slides are to be carefully cleaned before use with clean water and detergent followed by 95% ethyl alcohol polishing to remove every trace of grease that may spoil the results. Slides, particularly in warm tropical climates, should be stored packaged in a dry atmosphere to prevent contamination with dust and mould. Blood obtained by syringe for routine examination purposes may also be used to prepare specimens for malaria diagnosis, if no anticoagulant is used (Shute, 1988)[8].

If blood with anticoagulant is used, there is a 50% loss of parasites in 24 hours (Van den Ende, personal communication).

Sample preparation (thin film and thick film)

The microscopical observation of malaria parasites is optimal when parasites are fixed and observed in their natural location within red blood cells after appropriate staining. This is best accomplished with the *thin film* preparation technique. Unfortunately, thin film has a low sensitivity (100-200 parasites/µl of blood) and is thus inadequate for low parasitaemic infection. An adequate parasite concentration method is obtained by osmotic lysis of the red blood cells releasing the parasites, as is the case with *the thick* film preparation technique, the sensitivity of which is then increased (about 10 parasites/µl of blood). Experienced technicians may also prepare thick and thin films on the same slide (figure 1), even though particular attention is to be paid in this case to avoid fixation of the thick film (see below). Both methodologies are described below.

Thin film. One end of the slide is allowed to touch the top of the blood drop on the patient's finger. Only the top of the drop should come into contact with the slide. The quantity of blood to be transferred to the slide should not exceed 1.5 µl, usually corresponding to a diameter of 3-4 mm. The edge of a second slide (or a cover slip) is then laid on the drop of blood, that will spread on the entire line of contact between the two slides. The second slide, steadily held by the technician to form a 45° angle with the original slide, is then moved to the opposite end of the slide to which the drop was originally located. In the well prepared thin film, the blood film should end with multiple tails not touching the edges of the slide. Red blood cells should be visible one by one without overlapping. Abnormally thick slide may be the result of an exaggerated volume of blood or of an angle larger than 45°.

Thick film. A volume of blood twice to thrice the one used for a good thin film is needed for the preparation of a thick film (3.0 to 4.0 µl). This is usually accomplished by touching three times the top of the blood drop at the top of the finger to obtain a triangle on the slide (figure 1). The blood is then gently mixed for 20-30" using the corner of a second slide to defibrinate the blood and to obtain a round smear of about 1 cm in diameter. The thickness of the obtained film should allow the reading of the printed text of a usual newspaper through it (Gilles, 1993)[11].

Fixation and staining procedures

Thin and thick films may be allowed to dry in the air protected by dust, or actively dried by waving it. It is important to avoid abrupt exposure to heath (fire, sunlight) that may lead to fixation and fissure the preparations.

Thin films must undergo both processes of *fixation* and *staining*, while thick films must not be fixed to allow hemolysis of red blood cells and consequent dehemoglobinization. It is to be noted that fixation may also occur spontaneously (*autofixation*) with time (7 to 15 days, varying with humidity and temperature of the atmosphere). It is then important to process thick film as soon as possible to permit complete dehemoglobinization.

This chapter will review the more used fixing and staining procedures (Giemsa, Field, Leishman). Technical information on

the composition of staining and fixing solutions are given in Annex 2. For a more complete and detailed review, see Shute (1988)[8] and Gilles (1993)[11].

Fixation. Fixation may be achieved by heat and alcoholic solutions. Methanol (methyl alcohol) is the most widely used fixative for malaria thin films. Contact with methanol should be maintained for 10-20 seconds. If both thin and thick films are on the same slide, it is mandatory to avoid the contact of methanol (or even of its fumes!) with the thick film to avoid fixation.

Giemsa staining procedure. To achieve optimal results, it is important that the Giemsa staining solution is prepared with distilled or deionized water buffered to pH 7.2 (see Annex 2). The Giemsa staining solution is prepared by diluting Giemsa stain with buffered water A ratio 1:10 and 1:20 is usually used for staining thin and thick films respectively, that is 2 to 1 drops of Giemsa per ml of buffered water. Grossly, 5 ml of the solution is necessary to stain each single slide. The Giemsa stain is then poured onto the slide (or the slide is immersed in the staining trough) and contact is maintained for 30-40 minutes depending on the local climatic conditions. The staining solution is then removed by gentle but accurate rinsing with buffered distilled water (don't pour the stain, but wash it off!) and the slide is then drained on filter paper and allowed to dry in air. When the clinical situation requires immediate action, thick films may be stained with a Giemsa accelerate procedure. The qualitative morphological results are however far worse than the usual ones. Briefly, the thick smear is allowed to dry under a heat source of up to 50°C (even sunlight is suitable if slides are protected from dust) and then stained for 8 minutes with a 10% Giemsa solution (2 drops/ml) before rinsing and drying.

Giemsa staining is the most commonly used method for both thin and thick films all over the world for the quality of the stain and, of greater importance, its stability in tropical climates.

Field staining procedure. Field staining is a good method to stain thick films but is not suitable for thin films. However, it has the remarkable advantage to be extremely quick (the smear may be stained in less than 1 minute). Two different Field solutions (Field solutions A and B) are to be prepared (see Annex 2). Briefly, the slides are dipped first in Field solution A (3 seconds), then briefly drained by touching the edge of some filter paper and rinsed in distilled buffered water (3 seconds), dipped in Field solution B (1 second) and finally rinsed in distilled water to remove stain excess, drained and dried before observation.

Leishman staining procedure. Since Leishman staining solution uses methanol as a solvent, this method is only useful to stain thin films. Despite this drawback, the staining quality of the Leishman stain is excellent. Briefly, 7-10 drops of the staining solution (see Annex 2) are dropped on the slide. After 20 seconds, a few drops (10-15) of buffered water are also dropped on the slide and mixed with the stain by gently rocking. The staining mixture is allowed to rest on the slide for 20-30 minutes and then washed off by rinsing with buffered water before drying and observation.

Basic microscopy

The microscope is an essential component of the malaria diagnostic laboratory, even in field condition. The care and maintenance of the microscope is thus of the outmost importance. A basic knowledge of the main features of a microscope is also required (World Health Organization, 1991)[10]. Schematically, a light microscope is represented in figure 2. A monocular microscope is best suited for a natural solar light source, but the quality and the ease of observation is greatly improved when a binocular microscope (requiring electric light supply) is used. A routine microscope usually has three objectives (10x, 40x, 100x magnifications). The 100x objective is also referred to as "immersion objective" and has to be used to observe blood smears. The oculars also have their own magnification (6x or 7x are preferred for malaria diagnosis), the total magnifica-

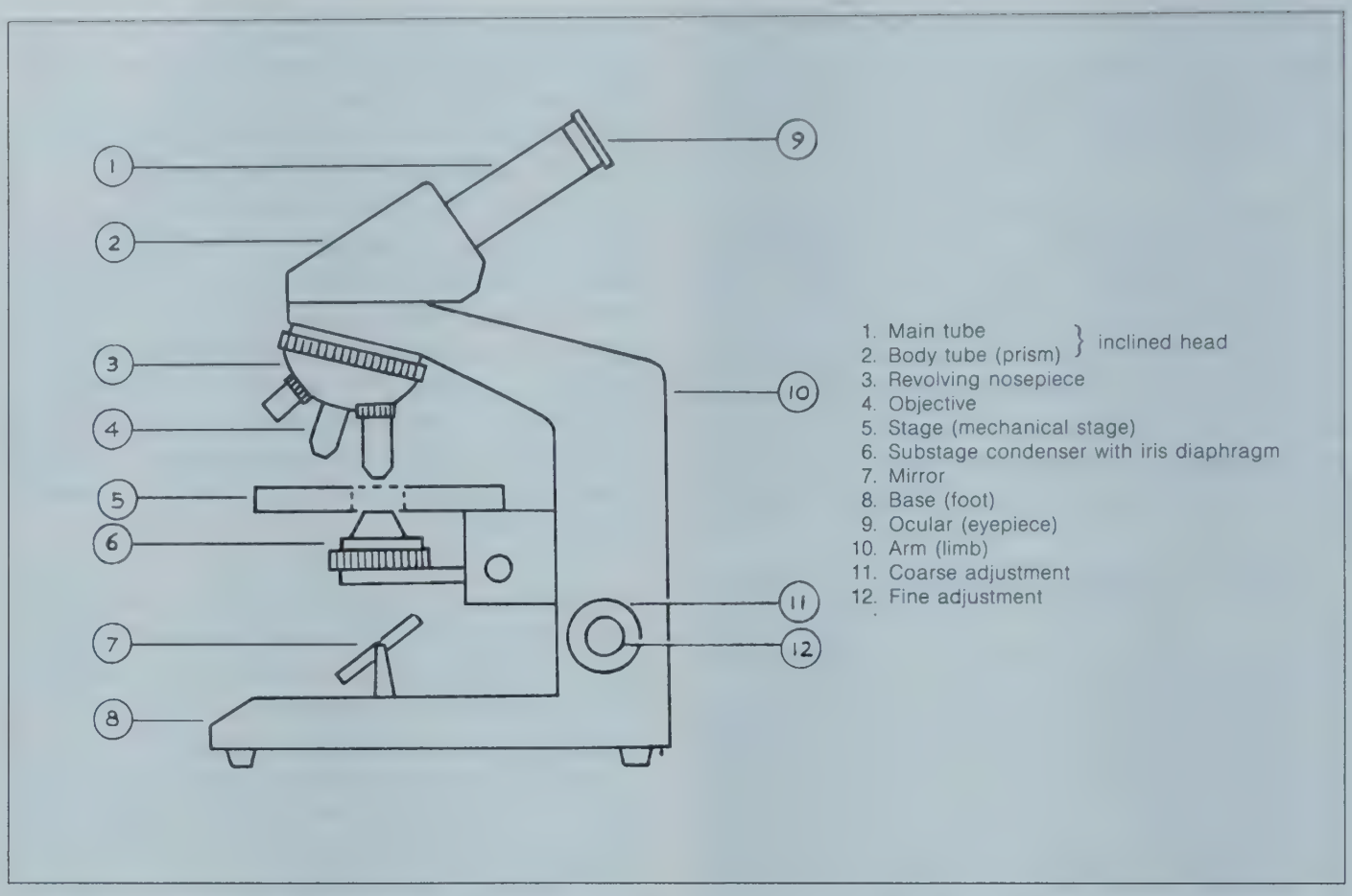


Fig. 2. Parts of a typical compound microscope (reproduced by permission of WHO, from: *Basic Malaria Microscopy. Part I. Learner's guide.* Geneva, World Health Organization, 1991).

tion resulting from the multiplication of both lenses.

The following practical suggestions may be of help for a better use of this invaluable diagnostic tool:

1. Place the slide on the stage;
2. Keep the diaphragm completely open and raise the condenser to reach the brightest field;
3. Using the coarse adjustment, lower the stage (or lift the objective, depending on the microscope used) to the maximum distance from the objective revolver
4. Place 1 drop of immersion oil on the slide;
5. Using the coarse adjustment, lift the stage to allow the oil drop to touch the lens of the objective;
6. Using the fine adjustment, sharply focus by slightly lowering the stage again;
7. Modulate iris diaphragm to obtain optimal illumination;
8. Clean the objective lenses with a soft gauze or lens tissue. Use xylene (always avoid strong alcohol solutions or acetone as these substances may dissolve the glue used to fix the objective lenses) only from time to time to remove traces of dried oil.

The observation: the four key questions

Four key questions need to be answered during the diagnostic itinerary to ascertain malaria infection:

1. Are malaria parasites present in the blood smear?
2. If yes, which species and stages do they belong to?
3. If yes, how many of them are present per l of blood?
4. Is this premunition or is this clinical malaria?

The answer to question n. 1 is better achieved by observing the thick film (especially in case of low level parasitaemia), while the answer to question 2 better requires the observation of thin films. Ques-

tion 3 may be answered by observing thick or thin films, depending on the technique used (see below). The following paragraphs will provide useful information to answer the key questions and allow an adequate qualitative and quantitative diagnosis of malaria infection. Question 4 will be addressed in detail in the following chapter on *Diagnosis of malaria disease*.

Morphological species and stage diagnosis

Complete knowledge of the morphological features of the different blood stages of the different *Plasmodia* species represents the essential basis of a correct laboratory diagnosis confirmation of malaria infection. It is to be stressed that a correct diagnosis may be done only after attentive and careful observation of a number of microscopic fields (at least 100 microscopic fields should be observed before a thick film may be classed as negative) and of a number of different morphologic characteristics that draw a well defined picture of the species. Do not rely on single images, even if apparently pathognomonic. A crescent-shaped gametocyte in peripheral blood does not obligatorily mean that a *P. falciparum* infection is the cause of the actual clinical complaint and, in any case, does not rule out the possibility of mixed infection!

This chapter will review the most peculiar features of each species of *Plasmodia* in both thin and thick film examination (Giemsa stain), providing information also on the most common artifacts that may deceive the unexperienced observer. A comprehensive synopsis of the morphological characteristics of the host cell and of the different stages of *Plasmodia* by species is provided in Tables 2 and 3. A graphic presentation of all *Plasmodia* stages is presented in Plates 1 to 4 and microphotographs showing thin and thick films are shown at the end of the chapter.

***Plasmodium falciparum* (Plate 1)**. In *P. falciparum* infection, only trophozoites and, in a later stage of the disease, gametocytes are found in the peripheral blood. The persistence of gametocytes even in treated patients is not surprising since the majority of antimalarial drugs are powerful schizonticides but weak gametocytocides and thus gametocytes may persist for weeks after the clinical attack. The observation of a schizont in peripheral blood is exceedingly rare, since schizogony usually occurs in red blood cell sequestered in visceral internal organs, and its occurrence is a sign of severe infection. At the onset of malaria attack, it is not uncommon to find trophozoites in different phases of maturation, reflecting the presence of different non synchronized broods of parasites entering the blood stream at different times. Usually, one brood prevails over the others and in few days synchronization takes place. *Plasmodium falciparum* penetrates both young and mature erythrocytes without preference.

In *thin blood films* the infected red blood cell is not usually enlarged and may show coarse red pigmentation only in mature trophozoite or schizont stages. *P. falciparum* trophozoites within red blood cells are ring-shaped and their dimension varies with age, the more mature the bigger. Young trophozoites may show the typical (and almost pathognomonic) presence of marginal forms ("*appliqué* forms"). Polyparasitized erythrocytes may be present and parasitaemia may be high. The presence of two purple red chromatin dots is not uncommon. When present, schizonts are compact, contain 15 to 30 nuclei and may have a single dark pigment mass in their context. Intraerythrocytic crescent-shaped *P. falciparum* gametocytes are blue with compact central coarse granules (female gametocyte) or pale violet, with scattered granules (male gametocyte). They may be seen in the second stage of the infection or in the convalescence phase.

In *thick blood films*, trophozoites assume various shapes (comma, ring, vibrio-like, dot, etc.) and are pale blue in colour, their size depending on the maturation stage. Gametocytes may also be seen in thick films and, when present, schizonts are usually distorted but multiple nuclei are easily visible.

***Plasmodium vivax* (Plate 2)**. In *Plasmodium vivax* infection, all stages of the schizogonic parasite developmental cycle may be seen in peripheral blood because schizogony takes place in circulating in-

	P. falciparum	*P. vivax*	*P. ovale*	*P. malariae*
Trophozoites	Always present in peripheral blood. Ring-shaped, small to medium size in dimension (Ø = 2-4 μm) depending on maturation. Young form may lay in marginal position. Polyparasitism and double chromatin dots possible.	Polymorphous in shape from large ring forms (younger forms) to ameboid mass occupying the entire red blood cell (mature forms).	Polymorphous in shape from ring forms often showing a central clear vacuole surrounded by regular cytoplasm (younger forms). to large ameboid masses (mature forms). Their dimensions are slightly inferior to *P. vivax.*	Ring form, small and regular in shape, with no pseudopodes. Older forms may be large, with vacuole. Occasionally, equatorial band form present.
Schizonts	Solely present in more severe infections. Small and compact, containing 15 to 30 merozoites and a dense dark brown pigmented residual body.	Normally present in peripheral blood. Large (Ø = 12-16 μm), round bodies containing 12 to 24 merozoites and loose golden brown residual pigmentation.	Normally present in peripheral blood. Large (Ø = 10-12 μm), round bodies containing 4 to 12 merozoites and dark pigmentation.	Compact, rosetta-like forms with 8-10 merozoites surrounding a central pigmented area.
Gametocytes	Present in the second phase of the erythrocytic cycle. Crescent-shaped with coarse rice-like granules and pigment. The female is blue in colour and granules are in central position, while the male form is violet and granules are scattered over the parasite.	Round regular bodies with a single voluminous nucleus (dense and red purple in female gametocytes, loose and pink in male forms).	Round regular bodies with a single voluminous nucleus (dense and red purple in female gametocytes, loose and pink in male forms). Their dimensions are usually inferior than in *P. vivax.*	Compact large single dense purple nucleus (female form) or loose violet nucleus (male) form). Scattered coarse pigment granules are present.
Parasitic density	May be very high (average 20-500,000, max 2,000,000).	Intermediate level (average 20,000, max 50,000).	Usually moderate (average 9,000, max 30,000).	Usually very low (average 6,000, max 20,000).

Table 2. Morphological features of the different stages of Plasmodia by species in stained thin blood films.

	P. falciparum	*P. vivax*	*P. ovale*	*P. malariae*
Age	Young and old erythrocyes infected.	Young erythrocytes infected.	Young erythrocytes infected.	Older erythrocytes infected.
Dimensions	Normal.	Enlarged.	Enlarged, sometimes assuming oval shape with *fimbriae* at one pole.	Normal.
Color	Normal to dark.	Normal to pale.	Normal.	Normal.
Granules	Unusual coarse scattered red stippling in mature trophozoites or schizonts (*Maurer's clefts*).	Frequent fine red diffuse stippling in all stages of erythrocytic developmental cycle (*Schuffner's dots*).	Frequent fine red diffuse stippling in all stages of erythrocytic developmental cycle (*Schuffner's dots*, also called *James' dots*).	None.
Pigment	Dark brown and usually compact.	Golden brown and usually loose.	Brown coarse pigment granules.	Brown coarse scattered pigment granules.
Leucocytes	The presence of malaria pigment in neutrophils and monocytes is a prognostic marker of severe disease (Hoan Phu *et al.*, 1995).			

Table 3. Morphological features of the host red blood cell by species of Plasmodia in stained thin blood films.

fected red blood cells non adherent to the endothelium of internal organs. In the early stages of the erythrocytic cycle, the possible presence of two or more non synchronized broods accounts for the concomitant presence of different maturative stages. *P. vivax* better penetrates younger erythrocytes, the natural deformability of which is essential to enable the blooming growth of the parasite.

In *thin films*, the host red cell is usually enlarged (up to twice the normal diameter) and is often filled with red fine membrane-derived granules (*Schuffner's dots*) that are extremely useful, when present, for identification purposes being present only in *P. vivax* and *P. ovale* infections. *P. vivax* trophozoites are extremely mobile due to the continuous emission of pseudopodi which confer the typical ameboid shape. Their dimensions increase as the parasite grows, sometimes to fill the entire red blood cell. In Giemsa stained thin blood films, the cytoplasm of *P. vivax* trophozoites is pale blue, while the chromatin dots (that may be double) are stained purple red. Schizonts are common in *P. vivax* infection and appear as large bodies containing 12 to 24 nuclei and a loose pigmented body. Gametocytes are also commonly seen and appear as round mononuclear bodies with a large nucleus (purple red and dense in female gametocytes, pink and loose in male forms).

In *thick films*, the Shuffner's dots (when present) may appear as an halo surrounding the parasite. *P. vivax* trophozoites appear as falsely fragmented cytoplasmic dots (joined by thin non visible cytoplasmic bridges) only one of which is provided with a voluminous purple red chromatin dot.

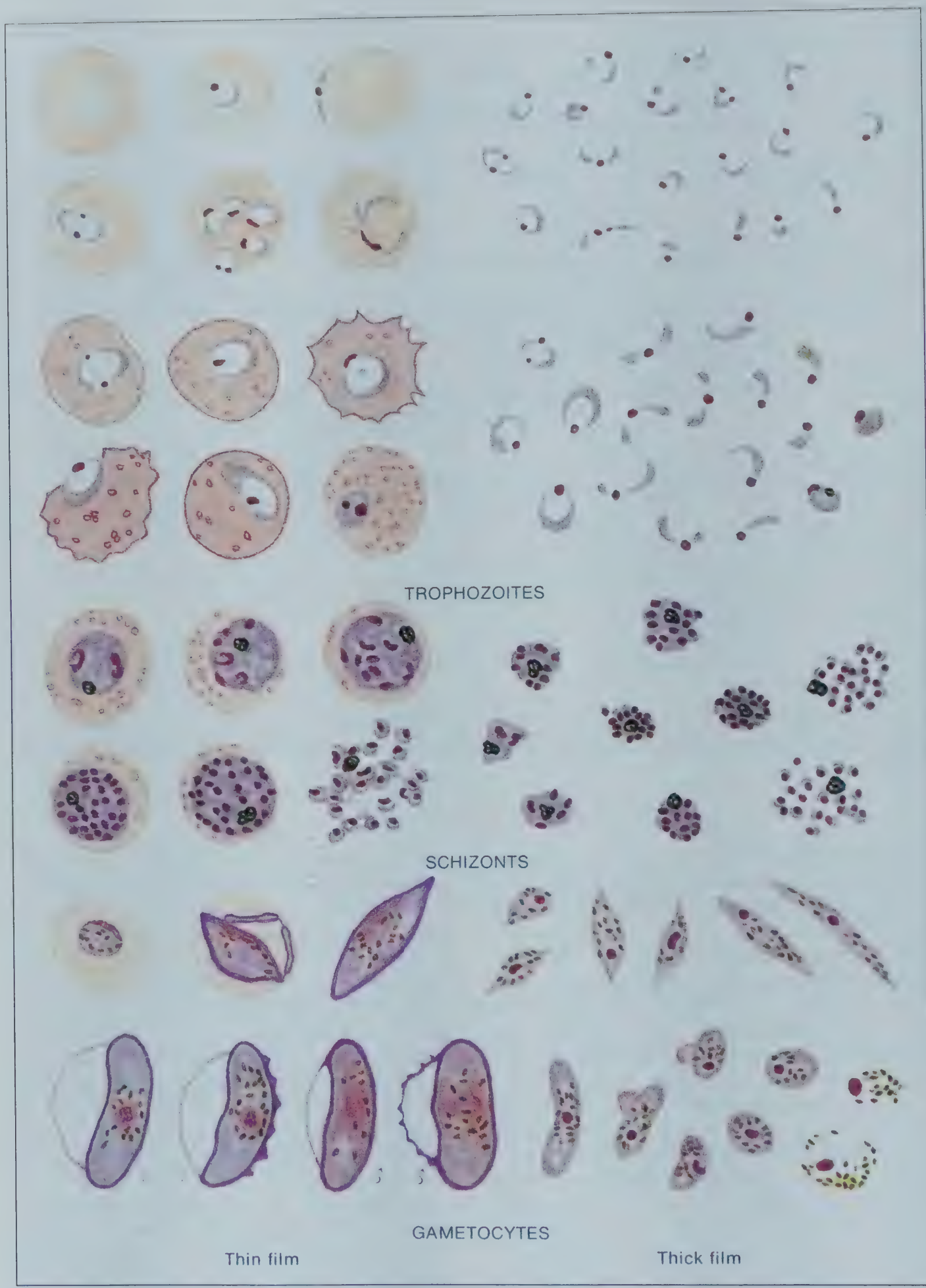


Plate 1. Appearance of *Plasmodium falciparum* stages in Giemsa-stained thin and thick films (reproduced by permission of WHO, from: *Basic Malaria Microscopy. Part I. Learner's guide.* Geneva, World Health Organization, 1991).

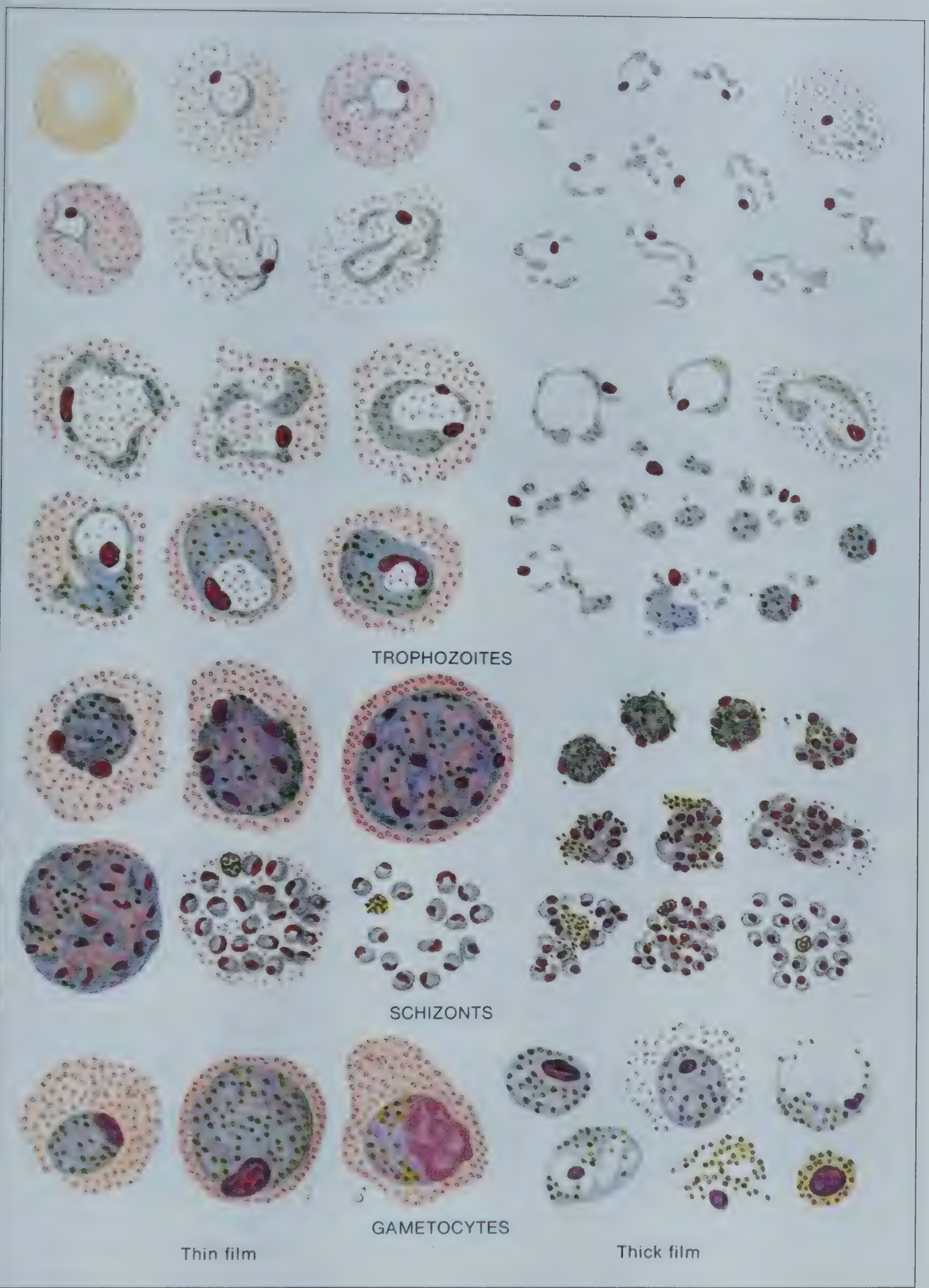


Plate 2. Appearance of *Plasmodium vivax* stages in Giemsa-stained thin and thick films (reproduced by permission of WHO, from: *Basic Malaria Microscopy. Part I. Learner's guide.* Geneva, World Health Organization, 1991).

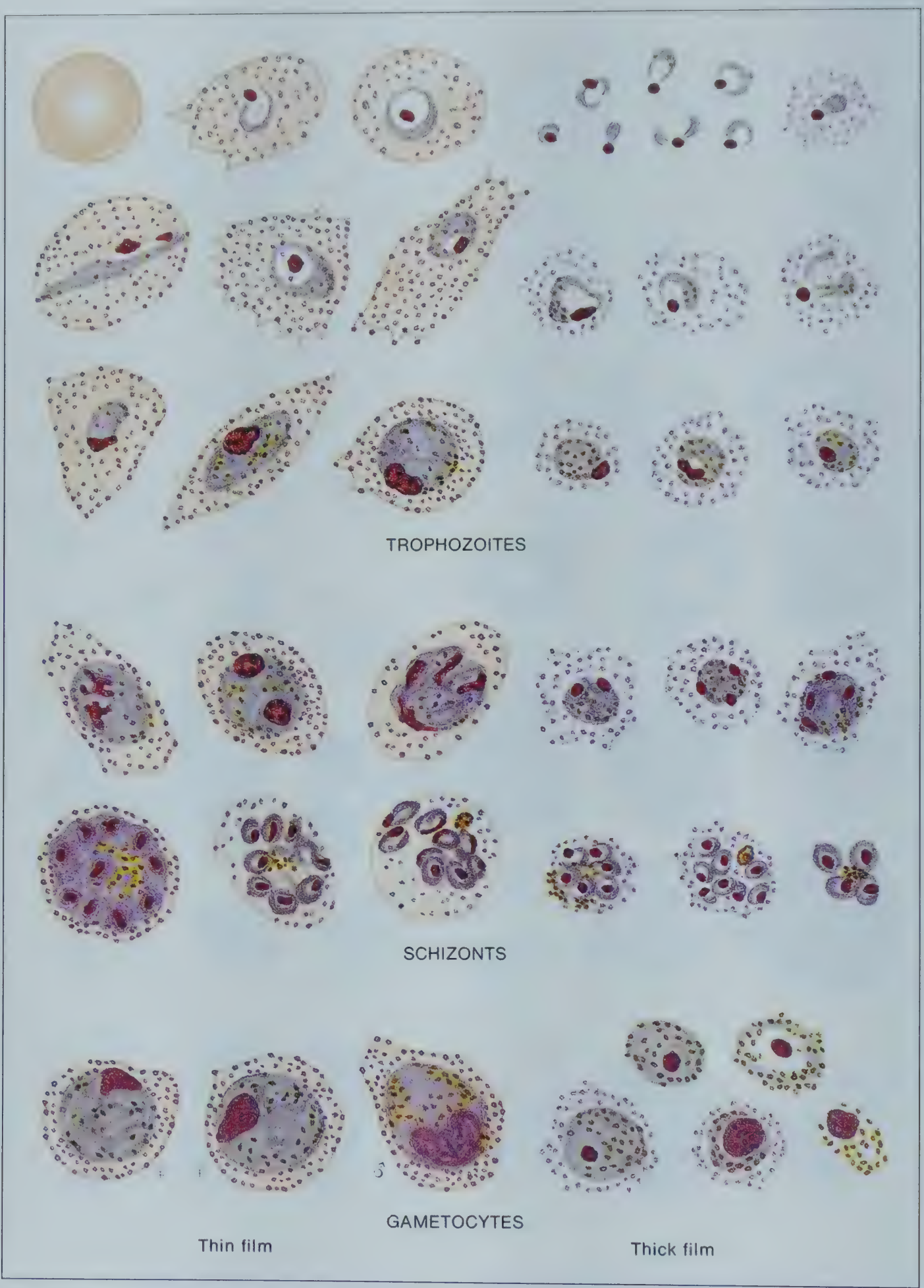


Plate 3. Appearance of *Plasmodium ovale* stages in Giemsa-stained thin and thick films (reproduced by permission of WHO, from: *Basic Malaria Microscopy. Part I. Learner's guide.* Geneva, World Health Organization, 1991).

***Plasmodium ovale* (Plate 3)**. As in *P. vivax* infection, to which its morphological and clinical features much resemble, all schizogonic developmental stages may be seen in peripheral blood in *P. ovale* infection. Trophozoites are ring to ameboid in shape and are contained in enlarged erythrocytes stippled with fine red dots (Schuffner's dots, also referred to as *James' dots* in *P. ovale* infection). The morphological appearance of schizonts and gametocytes is often also superimposable to the corresponding stage of *P. vivax*. The differential diagnosis between *P. ovale* and *P. vivax* infection (which is not always possible) is mainly based on the geographical setting (see Table 4 and chapter on "Epidemiology of human malaria Plasmodia" for further details) and on the peculiar oval shape with *fimbriae* at one pole that is occasionally, but not always, assumed by infected erythrocytes.

***Plasmodium malariae* (Plate 4)**. All schizogonic developmental stages may be observed in peripheral blood of subjects infected with *P. malariae*, whose merozoites preferably penetrate older red blood cells. No pigmentation is usually observed in the host erythrocytic cell, that are normal in size. Parasitaemic levels are usually much lower than in the other species of Plasmodia. Low level parasitaemia persisting for many years has been observed. Younger trophozoites are ring form, regular and small in size, with no pseudopodi. Occasionally, in blood smears, the trophozoites assume the typical equatorial band form. Older trophozoites may be irregularly ringed, occupying most of the host cell. Schizonts are compact, with 8-10 merozoites arranged in a rosetta-like fashion around the central pigmented area. Gametocytes are difficult to distinguish from late trophozoites and have coarse pigment granules scattered around the parasite body.

Artifacts. A number of possible artifacts may act as misleading factors for the unexperienced observer and particular attention and exercise is needed to rule out the possibility of confounding factors (figure 3).

The most frequent confounding elements are *platelets* which are superimposed on red blood cells. The absence of chromatin dots, the reddish stains and the different refrangent patterns while focusing are useful in differentiating platelets from malaria parasites. Among other possible misleading artifacts, *bacteria, dust particle, fungi spores, stain deposits* and *blood cell ghosts* are the most frequent

Quantitative parasitaemia count

The determination of the number of circulating parasites is exceedingly impor-

	P. falciparum	*P. vivax*	*P. ovale*	*P. malariae*
North Africa	Frequent	Predominant	Absent	Frequent
West Africa	Predominant	Very rare	Frequent	Rare
Central Africa	Predominant	Very rare	Rare	Frequent
East Africa	Predominant	Rare	Rare	Frequent
Indian Ocean	Predominant	Rare	Rare	Frequent
Central America	Frequent	Frequent	Absent	Rare
South America	Frequent	Predominant	Absent	Rare
Indian subcontinent	Frequent	Predominant	Very rare	Rare
South-East Asia	Predominant	Frequent	Rare	Rare
Pacific Islands	Frequent	Frequent	Rare	Rare

Table 4. Geographical distribution of the different species of Plasmodia in the tropics (from Basic Laboratory Techniques, WHO, 1982[13], modified).

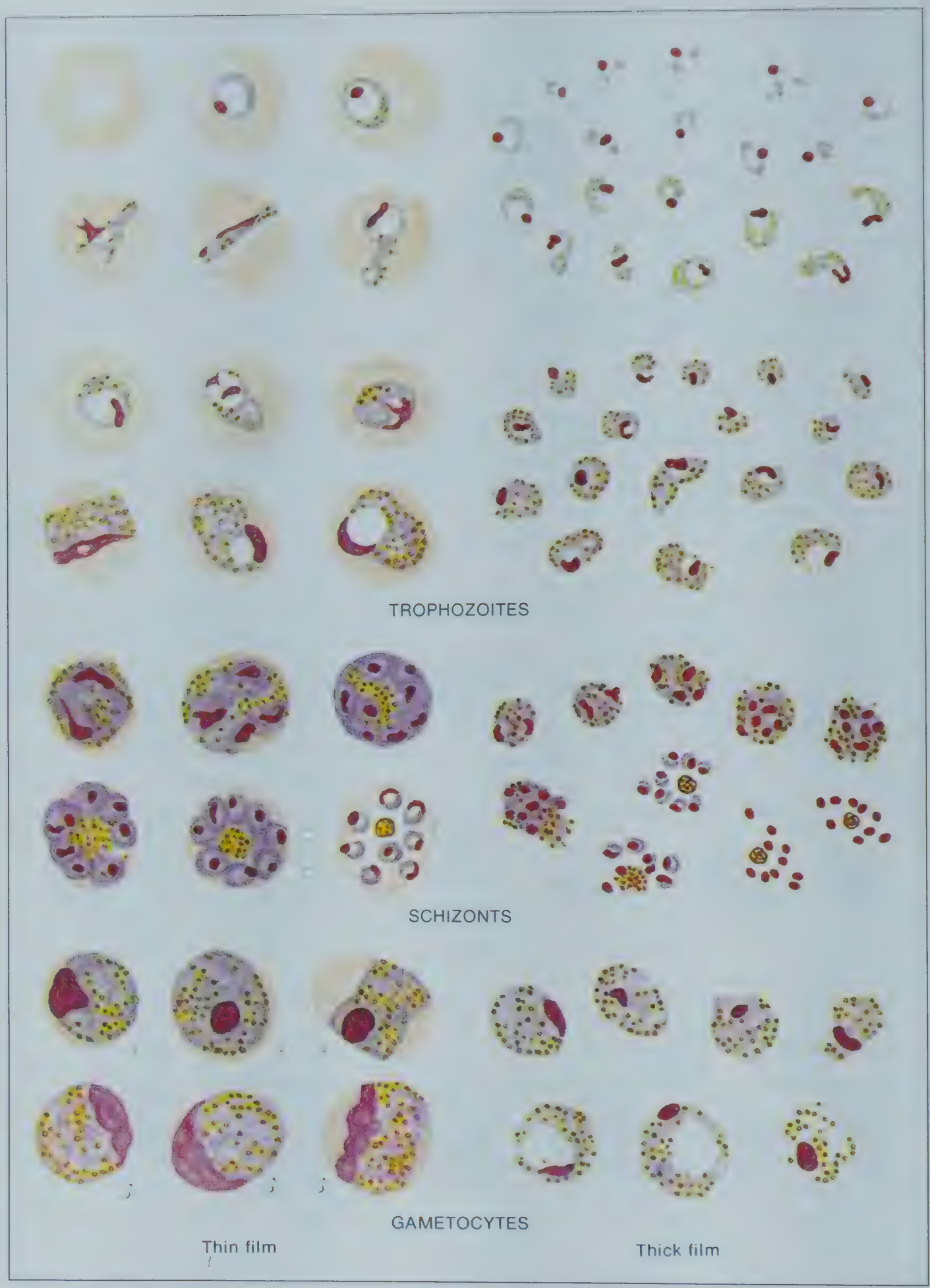


Plate 4. Appearance of *Plasmodium malariae* stages in Giemsa-stained thin and thick films (reproduced by permission of WHO, from: *Basic Malaria Microscopy. Part I. Learner's guide.* Geneva, World Health Organization, 1991).

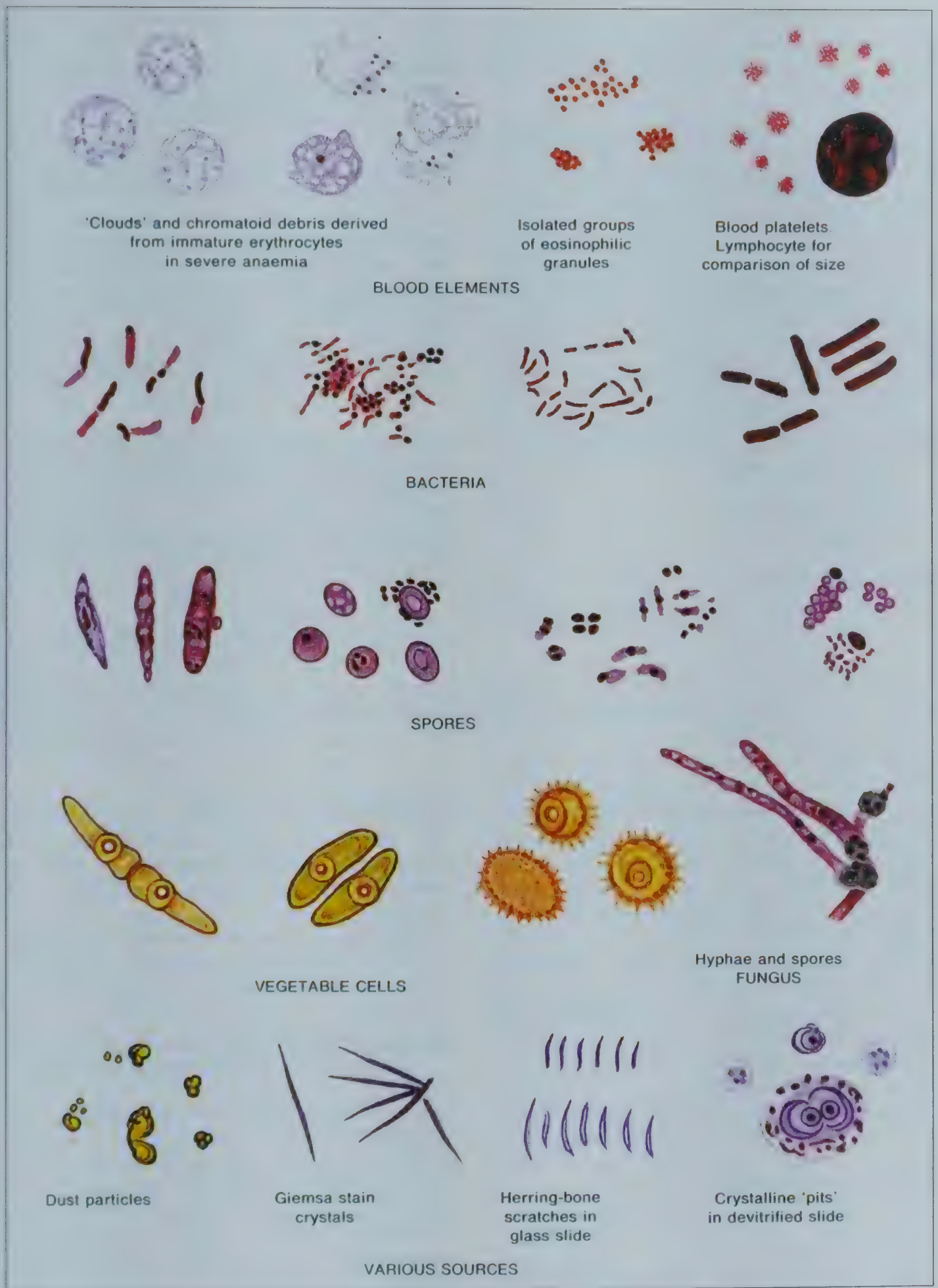


Fig. 3. Artifacts that may cause confusion in diagnosis (reproduced by permission of WHO, from: *Basic Malaria Microscopy. Part I. Learner's guide.* Geneva, World Health Organization, 1991).

tant for clinical purposes to monitor the evolution of the disease and the efficacy of therapy. Different methods have been proposed:

1) *Number of parasites/μl of blood (thick film)*. This method requires the observation of as many microscopic fields (100x) necessary to count 200 leukocytes. The number of asexual parasitic forms (trophozoites, schizonts) present in these microscopic fields has also to be recorded separately from the number of gametocytes. The calculation of the total number of parasites/μl of blood requires the knowledge of the total white blood cell (WBC) count value. If the haemogram is not available, the value of 8000 WBC is generally assumed (WHO, 1991)[10]:

$$\frac{\text{number of observed asexual parasites} \times \text{total WBC count/μl}}{200} = \text{parasites/μl of blood}$$

2) *Number of parasites/μl of blood (thin film)*. This method requires the preliminary determination of the number of erythrocytes present in the average microscopic field. This value is usually around two hundred, but it may vary considerably depending upon the quality of the smear and the magnification used. The actual red blood cell count (RBC) value is also needed. If the haemogram is not available, the value of 5.000.000 RBC/μl (males) and 4.500.000 RBC/μl (female) is generally assumed (Shute, 1988)[8] even though the frequent occurrence of anaemia consequent to malaria infection may render these estimations grossly inaccurate. The number of asexual parasites is then counted in at least 25 microscopic fields and the total parasite count/μl of blood is calculated as follows:

$$\frac{\text{number of observed asexual parasites} \times \text{total RBC count/μl}}{\text{total n. of RBC scanned in 25 microscopic fields}} = \text{parasites/μl of blood}$$

3) *Proportion of parasitized erythrocytes/total RBC count (thin film)*. This method requires the preliminary determination of the number of erythrocytes present in the average microscopic field. This value is usually around two hundred, but it may vary considerably depending upon the quality of the smear and the magnification used. The number of parasitized erythrocytes (asexual forms) present in 25 microscopic fields is counted divided by the total number of erythrocytes present in these fields, and multiplied by one hundred. The result will indicate the percentage of erythrocytes that are infected by malaria parasites.

4) *Semi quantitative count (thick film)*[10]. This method is fairly inaccurate and should be used only when it is not possible to perform one of the more accurate methods described above. It uses the following semi-quantitative scale:

+	1-10 asexual parasites per 100 thick film fields
++	11-100 asexual parasites per 100 thick film fields
+++	1-10 asexual parasites per single thick film field
++++	more than 10 asexual parasites per single thick film field

The commonest mistakes

A number of technical mistakes may hamper the correct diagnosis of malaria infection and are briefly listed below in Table 5.

Exercise and experience will teach the microscopist when one mistake has occurred during the preparation of the slide.

b) Quantitative Buffy Coat (QBC®) and the direct acridine orange staining

The Beckton Dickinson QBC® test is a sensitive microscopic test based on the ability of acridine orange to stain nucleic acid containing cells (Rieckmann *et al.*, 1989)[14]. Briefly, a microhaematocrit tube containing acridine orange stain and anticoagulant is filled with the patient's blood (55-65 μl), obtained by a conventional finger pricking method as described above. After insertion of a float, the microhaematocrit tube is centrifuged at 12.000 g for 5

Mistake	Result
Pricking on non accurately dried finger	The parasites and host cells may be fixed by the alcoholic detergent solution
Use of unclean slides	The blood smear will not be spread evenly
Excessive use of blood	The blood smear will not be spread evenly due to the beginning of the coagulation process
Too much blood is used for thin films	Erythrocytes are laid on multiple layers. Observation is impossible
Inadequate quantity of blood is used for thin films	Parasites may be virtually absent if parasitaemia is low
The slide is not immediately marked with the identification name or code	Confusion may arise leading to unidentified positive or negative slides
Use of incorrectly buffered water	Staining is poor. In particular, Schuffner's dots may not be evident
Use of hypertonic staining solution	Acantocytosis of erythrocytes
Drainage (and not washing) of stain from the slide	Stain deposits may render the observation difficult
Excessive time elapses before preparation and staining of thick films	Autofixation occurs and haemolysis is impossible
Exposure of thick films to excessive heath	Autofixation occurs and haemolysis is impossible
Thick films are dried too slowly	*P. falciparum* gametocytes may exflagellate

Table 5. Commonest technical mistakes in microscopic diagnosis of malaria.

minutes and immediately placed under an ultraviolet light source microscope for observation. The blood cellular components sediment in the tube so that platelets, lymphomonocytes, granulocytes and erythrocytes are separated and forced against the tube wall by the internal float.

The parasites are seen as fluorescent bodies standing at different levels of the sedimentation column depending on the stage and species of the parasite.

There is no consensus on the potential of the QBC® technique in replacing conventional microscopy in the field and many advantages and disadvantages have been pointed out at different times (Baird *et al.*, 1992; Anonymous, 1992; Bawden *et al.*, 1994)[15, 16, 17, 18]. Among the advantages, the *short time* and *ease of execution* (5-10 minutes) and the higher *sensitivity* than standard thick films at least in *P. falciparum* infections (as high as 3-4 parasites/µl of blood) are the most stricking ones (Rieckman *et al.*, 1989)[14]. Among the disadvantages, the *need for a fairly sophisticated instrumentation* (a 12.5x ocular and 50x objective and an ultraviolet light supplied microscope are recommended for optimal observation), *cost* (estimated at 1.5 ECU/test), the limited performance when *Plasmodia other than P. falciparum* are involved and the *difficulties in species determination and parasite quantification* are of concern (White N. J. and Silamut K., 1989)[19].

In our opinion, the QBC® technique is highly reliable and user-friendly (always coupled with conventional microscopy for species determination and quantification purposes) in a hospital setting and in the hands of well trained and experienced microscopists although it still needs to be validated for field use. QBC® should be used together, but should not replace, conventional standard thick and thin

blood films for malaria diagnosis under field conditions until its major drawbacks have been overcome.

A *direct acridine orange* (fluorochrome) staining of thin and thick films (see Annex 2) has been recently proposed to provide an economically convenient alternative to the QBC® technique for use in the field by using specially designed interference filters that may be connected to conventional light (even sunlight) microscopes (Kawamoto and Billingsley, 1992)[20]. No definite advantages over conventional Giemsa staining have however been ascertained so far (Delacollette and Van der Stuyft, 1994)[21]. Fluorochrome staining with *benzothiocarboxypurine* has also been proposed with claimed satisfactory results (Makler *et al.*, 1991)[22].

c) DNA probes and Polymerase Chain Reaction

Conventional microscopy is usually satisfactory for the large majority of clinical situations, but may be inadequate for very low parasitaemias (below the detection threshold of 10 parasites/µl of blood) and to detect drug-resistant parasites. The possibility that modern molecular biologic techniques overcome these drawbacks has thus been explored. The initial studies in nucleic acid-based malaria diagnosis used the parasite's ripetitive DNA found throughout the *Plasmodium* genome as the diagnostic target (Franzen *et al.*, 1984)[23]. Therefore, after the sequencing of two small subunits (18S) rRNA genes from *P. falciparum* and *P. vivax* (Mc Cutchan *et al.*, 1988; Waters *et al.*, 1989)[24, 25], species-specific regions of the rRNA genes have been exploited in developing a sensitive and specific diagnostic procedure. In fact, ribosomal RNA probes are considered to have greater potential than DNA probes since the RNA content in *Plasmodium* is one and a half times the amount of DNA and rRNA represents 85-95% of total cellular RNA. Once the Polymerase Chain Reaction technique became widely described, several investigators switched to designing PCR assays to detect *P. falciparum* and *P. vivax* because of the inherent increase in analytical sensitivity compared to DNA-RNA probes assays.

The PCR based method to diagnose parasitic infection has been recently reviewed (Weiss, 1995)[26].

PCR characteristics

Several PCR assays to detect Plasmodium DNA in human blood by using different approaches to increase sensitivity and specificity have been described. The PCR can detect a single *P. falciparum* in 20 µl of human blood when primers based on moderately-repetitive DNA probe pBRK1 are used (Tirashopon *et al.*, 1991)[27], 100-fold more sensitive than the standard thick blood film. Therefore, an improvement on sensitivity was obtained using olygonucleotides complementary to the 18S rRNA genes (Snounou *et al.*, 1993)[28]. The PCR-based techniques have also proven highly species-specific, a quality which is particularly useful in clinical practice where microscopically undetected mixed infections may be demonstrated by PCR (Brown *et al.*, 1992)[29]. Another very interesting and recently described characteristic of PCR based techniques is the possibility of processing dried blood spots collected on filter paper, without the need for difficult laboratory steps in the field, even 6 months after collection. This method may reduce the risk of contamination in the laboratory and has yielded satisfactory sensitivity results for *P. falciparum* (Long *et al.*, 1995)[30].

PCR applications

The high sensitivity of PCR based techniques has been applied to the *screening of donor blood* with promising results and relatively acceptable costs in Viet Nam (Hang *et al.*, 1995)[31] and other Developing Countries. In this application, the possibility of false positive results is not a major concern because it implies the discharge of few donor blood units compared to a substantially lower incidence of transfusion malaria cases from asymptomatic low parasitaemia donors in highly endemic areas.

An other area of major interest where PCR based techniques may play an important role is the *epidemiological study* of the distribution of different Plasmodia (mainly *P. falciparum*) isolates within the

insect and human hosts in a given geographical area, so providing intimate information on the biology of the parasite and pathogenesis of the disease in the population at risk (Viriyakasol S *et al.*, 1995)[32]. Finally, it is known that *P. falciparum drug resistance* to different antimalarial drugs is due to point mutation in different amino acids (i.e. amino acid 108 in dehydrofolate reductase is responsible for resistance to antifolate drugs). The possibility to discriminate drug-resistant *P. falciparum* strains from drug-sensitive ones without the need for labour-intensive culture has already been demonstrated (Peterson *et al.*, 1991)[33].

PCR drawbacks

Nevertheless, a number of drawbacks hamper the use of the PCR technique for the diagnosis of malaria infection as well as of other viral, bacterial or parasitic infections not only in the field but also in well equipped laboratories in industrialized countries, the most important of which being contamination, yielding false positive results, and high costs. Stringent, extensive and costly precautions (rarely obtainable in real field conditions) are required to diminuish the rate of contamination of PCR with previously amplified products (Wilson, 1993)[34]. Finally, actual costs confine the polymerase technique to selected laboratories, mainly in industrialized malaria-free Countries for research purposes.

It is hoped that the new biotechnological advances will soon offer a real inexpensive, sensitive and user-friendly PCR-based tool to be used in real field conditions for clinical, preventive and epidemiological purposes.

A detailed review of the technical features of Polymerase Chain Reaction (PCR) based malaria diagnostic techniques far exceeds the aims of this Handbook and will not be addressed here.

d) Detection of *P. falciparum* antigen

Contrary to the presence of antibodies, the presence of parasitic specific *antigens* may reflect active infection. The methods used to detect antibodies have also been adapted to the detection of antigens, with variable results. Recently, the advent of biotechnology has disclosed new horizons in the field of antigen detection. A brief outline of the most promising techniques is reported below. The production of histidine-rich protein II (HRP-II) antigen by blood stages of *Plasmodium falciparum* (Parra *et al.*, 1991)[35] forms the basis for the development of ELISA antigen test (Taylor and Voller, 1993)[36] and, more recently, of the dipstick *Becton Dickinson ParaSight ®-F test*, not needing microscopy for its execution, that has recently been proposed for the field diagnosis of malaria (Shiff *et al.*, 1994)[37]. The latter test has shown promising results and is dealt with in some detail below. After the deposition in a plastic well, a drop of blood is haemolysed by a detergent and put into contact with a anti-HRP-II monoclonal antibody coated dipstick. In the case of a positive reaction, the latter addition of anti-HRP-II monoclonal antibody conjugated with sulpho-rhodamine B as a marker produces a red coloration at the site of immunological reaction (a red line appears one cm from the bottom of the dipstick) after few minutes. The test is simple, quick to execute (11 minutes according to the manufacturers), stable for months even in warm and humid climates and does not need trained specialized personnel or equipped laboratories, as recently demonstrated by village health workers in rural Tanzania (Premji *et al.*, 1994)[38] and by field malaria control activities in Brazil (Dietze *et al.*, 1995)[39]. These characteristics makes the ParaSight ®-F a good candidate for malaria diagnosis in the field or in blood bank in endemic areas where a large number of tests are to be carried out daily, even though costs are far higher than conventional microscopy. The test may also be of oustanding value in clinical settings in non endemic areas. The sensitivity of the test nevertheless decreases linearly with the decline of parasitaemia (from 96-100% in specimens with more than 60 *P. falciparum* parasites/µl to 11-67% in specimens with 10 parasites/µl or less) (Beadle *et al.*, 1994)[40] and is useless in the detection of *Plasmodia* infection other than *P. falciparum.* Furthermore, the test does not permit quantitative determination (useful

in highly endemic areas where subclinical parasitaemia is common) and may give false positive results some day after therapy-induced clearance of parasites from the blood due to the persistence of the histidine-rich protein II in the blood.

e) Other direct diagnostic methods

The determination of blood levels of parasite-specific lactate dehydrogenase (pLDH) has been evaluated as an indirect method of quantifying parasitaemia and also drug resistance (Makler and Hinrichs, 1993)[41]. The method may be automated allowing the processing of large numbers of samples, but its sensitivity has proven to be quite low (Knobloch and Henk, 1995)[42].

Indirect parasitological diagnosis

The limitation of conventional microscopy (low sensitivity, labour intensive, well trained personnel needed) and of the new diagnostic direct techniques (sophisticated instrumentations required) has stimulated the research for immunodiagnostic methods that might couple the advantage of easy processing a large amount of samples in a standardized way. The detection of Plasmodia specific antibodies is briefly delineated below.

Detection of Plasmodia specific antibodies

The presence of the malaria parasite in the patient's organism elicits the production of a wide range of antibodies, both specific against Plasmodia antigens and non specific against leukocytes, red blood cell, rheumatoid factor, etc. (Ferreira, 1990)[43]. Stage-specific, species-specific and genus-specific antibodies are detectable in the blood some days after parasite invasion of the bloodstream and may persist for long periods of time after infection has occurred (from a few months in case of short-lived and therapy-terminated infection to many years in case of chronic or relapsing infection) (Voller, 1988)[44] (figure 4).

Antibodies against all blood stages of the parasite schizogonic cycle, and even against exoerythrocytic schizonts, have been demonstrated but commonly available serological tests are aimed at the detection of antibodies against asexual blood stages for practical purposes (supply of antigen) (Voller, 1988)[44]. The best antigens are obviously homologous antigens (*P. falciparum, P. vivax, P. ovale, P. malariae*), but an adequate supply is practically obtainable only for *P. falciparum*, which is cultured *in vitro*. For the serological diagnosis of the other species of Plasmodia, heterologous antigens are to be used (*P. cynomolgi* for *P. vivax* and *P. brazilianum* for *P. malariae*).

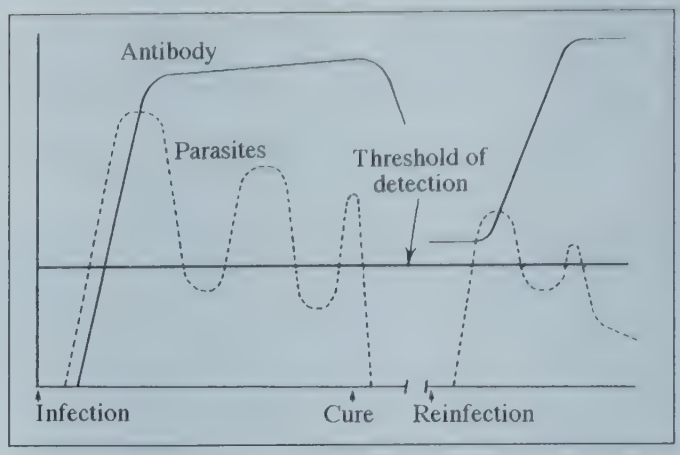


Fig. 4. Evolution of parasitaemia and antibodies in malaria (immunofluorescence) (Amended from Voller and Draper, 1982)[45].

The first serological test to be used for malaria antibodies was immunofluorescence (IFAT), which may give quantitative results for both G and M specific immunoglobulins. Its specificity and sensitivity largely rely on the laboratory technician's expertise. Results higher that 1:20 are considered to be positive and high titers (> 1:200) probably reflect recent infection. The indirect haemoagglutination test (IHA) is simple and suitable for field studies, but its sensitivity and specificity are poor. The enzyme-linked immunosorbent assay (ELISA) has similar sensitivity and specificity characteristics than the IFA test, but the interpretation of the results may be better standardized. Finally, for research purposes, radioimmunoassay (RIA) is sometimes used but needs well equipped research laboratories and personnel.

The characteristics of the different immunologic methodologies used to detect Plasmodia specific antibodies are summarized in Table 6.

Test	Current application	Notes
Indirect Immunofluorescence (IFAT)	Diagnosis of infection Epidemiological studies	Erythrocytic schizonts as antigens Fair sensitivity and specificity Fluorescent microscope needed Trained personnel needed Difficult to standardize
Indirect haemoagglutination (IHA)	Epidemiological surveys	Low sensitivity and specificity Simple material needed Suitable for field conditions
Enzyme-linked immunosorbent assay (ELISA)	Diagnosis of infection Epidemiological studies	Fair specificity, sufficient sensitivity Possible in field conditions
Radioimmunoassay (RIA)	Research studies	Expensive Sophisticated instruments needed

Table 6. Currently used immunological tests to detect Plasmodia specific antibodies (from Gilles, 1993, adapted)[11].

Since the presence of specific antibodies only reflects past infection, it is obvious that seroprevalence rate is very high in populations living in malaria endemic areas, where it linearly increases with age. The detection of specific antibodies has therefore little role to play in the individual diagnosis of actual clinically relevant infection in malaria endemic areas.

Application of immunological methods

Though the protective importance of antibodies to prevent or decrease the clinical expression of malaria symptoms and signs has been established, their role in the individual diagnosis of clinical feverish episodes is now considered poor, at least in endemic areas.

Immunodiagnosis may provide useful informations in the following fields of application:

1) *Diagnosis of clinical attacks*. The detection of specific antibodies has no role in the individual confirmatory diagnosis of suspected clinical attacks in endemic areas. Outside endemic areas, it may retain some clinical utility only in ruling out malaria in microscopically negative feverish patients returning from short trips to malaria endemic areas. This is particularly true for patients who took chemoprophylaxis or self-treatment during the feverish attack abroad without diagnostic confirmation.

2) *Epidemiological studies*. Conventional antibody serology has provided exceedingly useful species-specific epidemiological information in malaria control programmes to define malaria transmission areas and monitor effectiveness of preventive intervention strategies. The possibility of processing easily large numbers of samples and to evaluate the past exposure to the infecting agent (microscopy, PCR and antigen detection only point out present prevalence of infection) are important factors that support the use of conventional serology for epidemiological purposes. The detection of the highest transmission altitude in Africa and of the presence of residual transmission foci in Tunisia after control activities, the confirmation of malaria eradication from Mauritius and Greece, the validation of chemoprophylactic malaria control in Panama are only a few examples of its possible applications in epidemiological studies (reviewed in Voller, 1988).

3) *Donor blood screening*. Transfusion malaria is one of the most dreaded public health problems in highly endemic malaria Countries sometimes occurring also in malaria free western Countries. Mi-

croscopy is an unsuitable screening method in malaria-free areas since low parasitaemia is usually undetectable and long performing time is needed. In these areas, the "five year rule" (all persons reporting stays in malaria endemic areas will be excluded from donation for a five year period) is safe, but the increasing number of travels to the tropics for touristic and commercial reasons will probably render it too stringent in the coming years. Serology will then provide a good alternative to evaluate the persistence of low level malaria parasites in non endemic areas. On the contrary, serology has no role in blood screening activities in malaria endemic areas where continuous exposure occurs.

Acknowledgements

The Authors wish to thank Dr Maurizio GULLETTA for his skilfull technical assistance in preparing microphotographs.

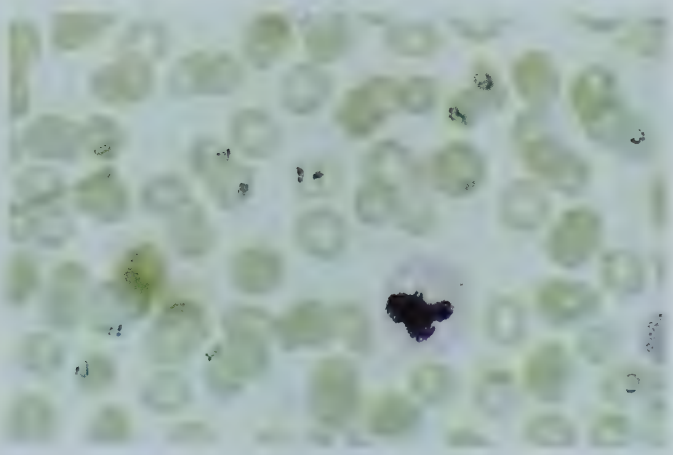


Fig. 5. Thin film. *P. falciparum* young trophozoites in red blood cell. Note the presence of marginal forms and polyparasitized cells.

Fig. 6. Thin film. *P. falciparum* typical crescent-shaped gametocyte.

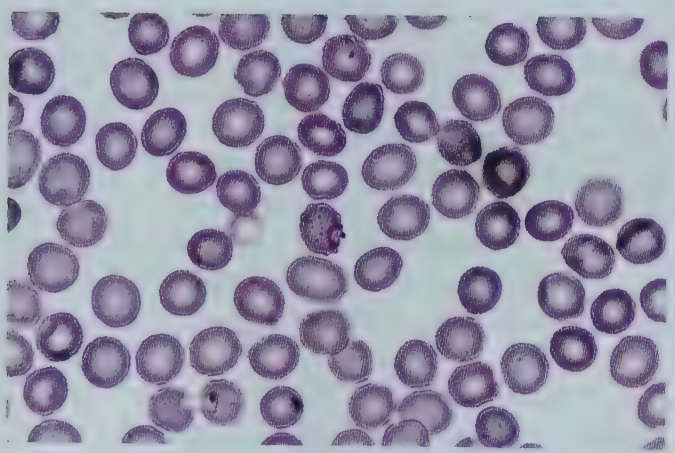


Fig. 7. Thin film. *P. falciparum* trophozoite in red blood cell that is filled with coarse red stippling (Maurer's clefts).

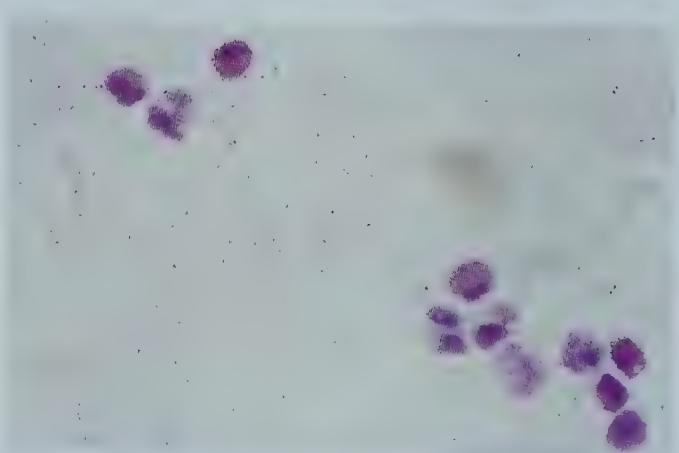


Fig. 8. Thick film. *P. falciparum* highly parasitaemic (> 100.000 µl) blood sample.

Fig. 9. Thick film. *P. falciparum* crescent shaped gametocyte.

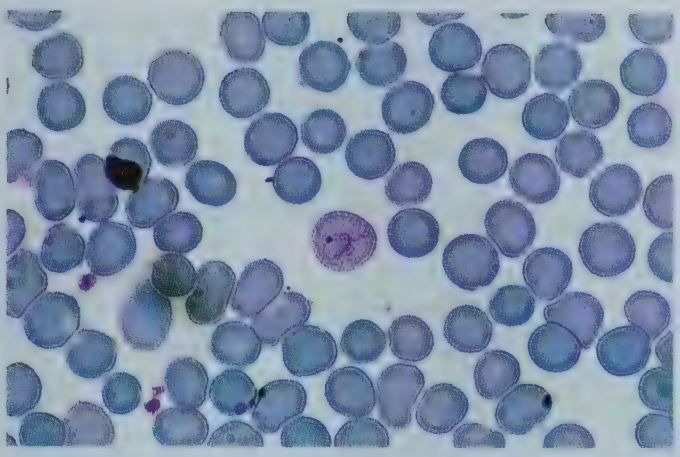


Fig. 10. Thin film. *P. vivax* trophozoite. Note the fine diffuse stippling inside the cytoplasm of the enlarged red blood cell (Shuffner's dots).

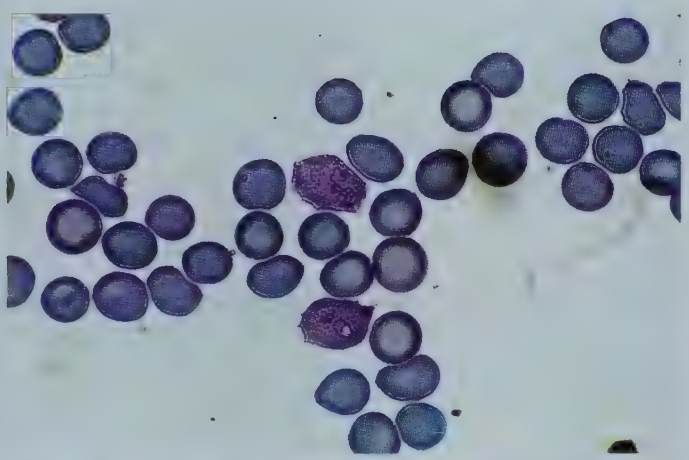


Fig. 11. Thin film. *P. ovale* trophozoites within elongated red blood cell with polar *fimbriae*.

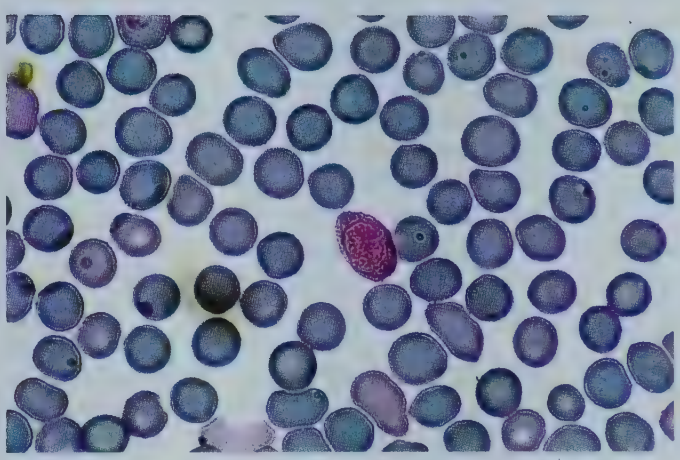


Fig. 12. Thin film. *P. ovale* gametocyte.

Fig. 13. Thin film. *P. malariae* trophozoite. Note the equatorial band form of the parasite within the normal red blood cell.

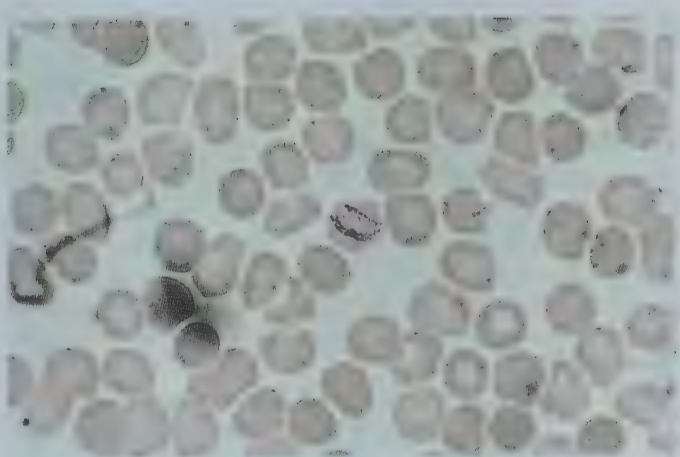


Fig. 14. Thin film. *P. malariae* rosetta-like schizont with pigment.

Annex 1 - Laboratory material needed for light microscopical observation of thin and thick films for malaria diagnosis

Optic microscope with 100x oil immersion objective
Electric supply of adequate mirrors for sun light
Stain stock solution
Buffered distilled/deionized water
Graduated cylinders
Staining recipients
Clean glass slides
Cover slips (optional)
Disinfectant alcoholic solution
Sterile pricking needles
Gauze or cotton

Annex 2 - Stock stain solutions for malaria microscopy

After: Shute G. T. **The microscopic diagnosis of malaria**. In *Malaria. Principles and practice of malariology* (Wernsdorfer W. H., McGregor I. eds), 1988: 781-814[8]
World Health Organization. **Basic Laboratory Techniques**. World Health Organization, Geneva (Switzerland), 1982[13]

a) Field stock solution

Solution A

Medicinal methylene blue	*0.8 g*
Azur I	*0.5 g*
Disodium hydrogen phosphate anhydrous Na_2HPO_4	*5.0 g*
Potassium dihydrogen phosphate KH_2PO_4	*6.25 g*
Distilled water	*500 ml*

Solution B

Eosin	*1.0 g*
Disodium hydrogen phosphate anhydrous Na_2HPO_4	*5.0 g*
Potassium dihydrogen phosphate KH_2PO_4	*6.25 g*
Distilled water	*500 ml*

For both solution A and B, prepare first the phosphate solutions and pour them into a hard glass bottle containing glass beads. Add the stains and mix accurately. The stock solution needs to be filtered every two weeks and to be stored in tightly stoppered bottles to avoid the growth of moulds. Solution A and B may also be prepared from the commercially available (Field A powder 5.0 g must be accurately dissolved in 600 ml of hot water and Field B powder 4.8 g must be dissolved in 600 ml of hot water).

b) Giemsa stock solution

Giemsa stain (powder)	*3.8 g*
(Giemsa Azur II 0.8 g + Azur II eosin 3.0 g)	
Methanol	*250 to 300 ml*
Glycerol	*200 to 250 ml*
	(to total 500 ml)

Mix in a clean hard glass bottle containing about 50 glass beads and shake vigorously for a few minutes. Repeat shaking each day for 4 days before using the solution.

c) Leishman stock solution

Leishman powder	*1.5 g*
Methanol	*1000 ml*

The powder and the methanol are mixed in a clean hard glass bottle containing glass beads.Screw the cap tighly to avoid humidity and mix accurately.

d) Acridine orange stain for thick and thin films

Acridine orange	Sigma Chemicals n. A-4921

See Kawamoto F., Billingsley P.F. Rapid diagnosis of malaria by fluorescence microscopy. *Parasitology Today*, 1992; 8: 69-71 for further details on the technical procedure of staining thick and thin films.

e) Buffered water

Stock solution I

di-sodium hydrogen phosphate	9.5 gr
distilled water	1000 ml

Stock solution II

potassium di-hydrogen phosphate	9.07 gr
distilled water	1000 ml

To obtain one liter of buffer solution, mix the 2 solutions as follows:

pH 6.8 = Sol I (ml 68.4) + Sol II (ml 126.8) + distilled water (ml 804.8)
pH 7.0 = Sol I (ml 89.5) + Sol II (ml 99.2) + distilled water (ml 811.3)
pH 7.2 = Sol I (ml 105.0) + Sol II (ml 77.2) + distilled water (ml 817.8)

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DIAGNOSIS OF MALARIA DISEASE

J. Van den Ende, L. Lynen

Introduction

Confirming malaria infection does not automatically mean that the patient's symptoms are due to this infection. In non-immune patients, this is highly probable, but in endemic countries many otherwise healthy people can have parasites on their blood slides. In this setting, symptoms might be caused by another disease. How to distinguish then between "malaria infection" and "malaria disease"?

This chapter will first describe a method to determine malaria morbidity in endemic settings, and discuss the diagnostic pitfalls related to the difference between "malaria disease" and "malaria infection". Based upon this difference clinical presumptive diagnosis will be put in perspective. In addition we discuss additional laboratory investigations, and we mention briefly the differential diagnosis starting from the key symptoms and signs.

What is malaria disease?

The diagnosis of malaria disease can be difficult. Let's start with some examples where making the correct diagnosis was a problem in the authors' experience.

One day in Zaire in 1976, one of us was called to the 1-year-old daughter of a colleague, who had fever and a convulsion. She was on daily chloroquine malaria prophylaxis. She was brought to the clinic, where she had another convulsion that ended in unrousable coma. Respiratory arrest occurred. Mouth to mouth resuscitation was started, a thick film was taken, and IM quinine was immediately given. Shortly afterwards, cardiac arrest occurred, but with cardiorespiratory resuscitation cardiac rhythm was quickly restored and spontaneous respiration resumed after half an hour. The thick film was reported to be negative. For years we have discussed whether this was cerebral malaria or not.

A second case in the same region was a 32-year-old local chief, who presented one evening in 1984 with abnormal behaviour and a fever of 38.5°C. A thick film showed scanty parasites of P. falciparum, chloroquine was given. The next day the patient was well. The diagnosis of incipient cerebral malaria was made. Several months later, the patient presented again, this time with extensive Kaposi lesions, and he died with the diagnosis of AIDS a short time later.

A third case was a 10-year-old child in Guinea-Conakry, who was seen with fever at the health centre. Chloroquine was given, but after three days the fever did not disappear, and the parents decided to go to the district hospital. The child was hospitalised, a thick film was taken, and chloroquine was again given. The next day, the result of the thick film arrived: it

was scanty positive for P. falciparum. Chloroquine was stopped, and quinine was administered, supposing a chloroquine resistant strain. After three days, the fever did not resolve, the diagnosis of typhoid fever was made on clinical grounds and chloramphenicol was given. The next day the child complained of pain in the right subcostal region. An ultrasound showed a liver abscess, probably amoebic.

Are the diagnoses and the decisions correct in all these cases? Before we discuss the cases, some general notions have to be explained (or gone through again).

Malaria disease versus malaria infection

What is malaria disease? We could state that "malaria disease" is the presence of symptoms and signs provoked by the parasite. The difference between malaria disease versus malaria infection is more than academic. Since parasites can be found in a high proportion of apparently healthy people in endemic regions, the mere presence of parasites is not a proof of a causal relationship between these parasites and the symptoms and signs. Moreover, symptoms and signs are not specific to malaria, we lack an unequivocal specific *indicator of morbidity* of malaria.

Thresholds

Parasitological threshold

The number of parasites present in a microlitre of blood, required for the technician to be able to detect them in a thick film or with the other diagnostic tools (see chapter "Diagnosis of malaria infection") is the *parasitological threshold* (figure 1). It depends on the type of laboratory technique, the time spent for searching parasites, the skill of the technician. For a thick film, it is generally accepted to be as low as 1 parasite per microlitre, but good laboratories go a lot further for suspected cases (T. Vervoort, personal communication). It should be stressed that this threshold does not depend on the patient.

Clinical threshold

The transition from the presence of parasites (triggering and sustaining immunity), to symptoms unequivocally attribut-

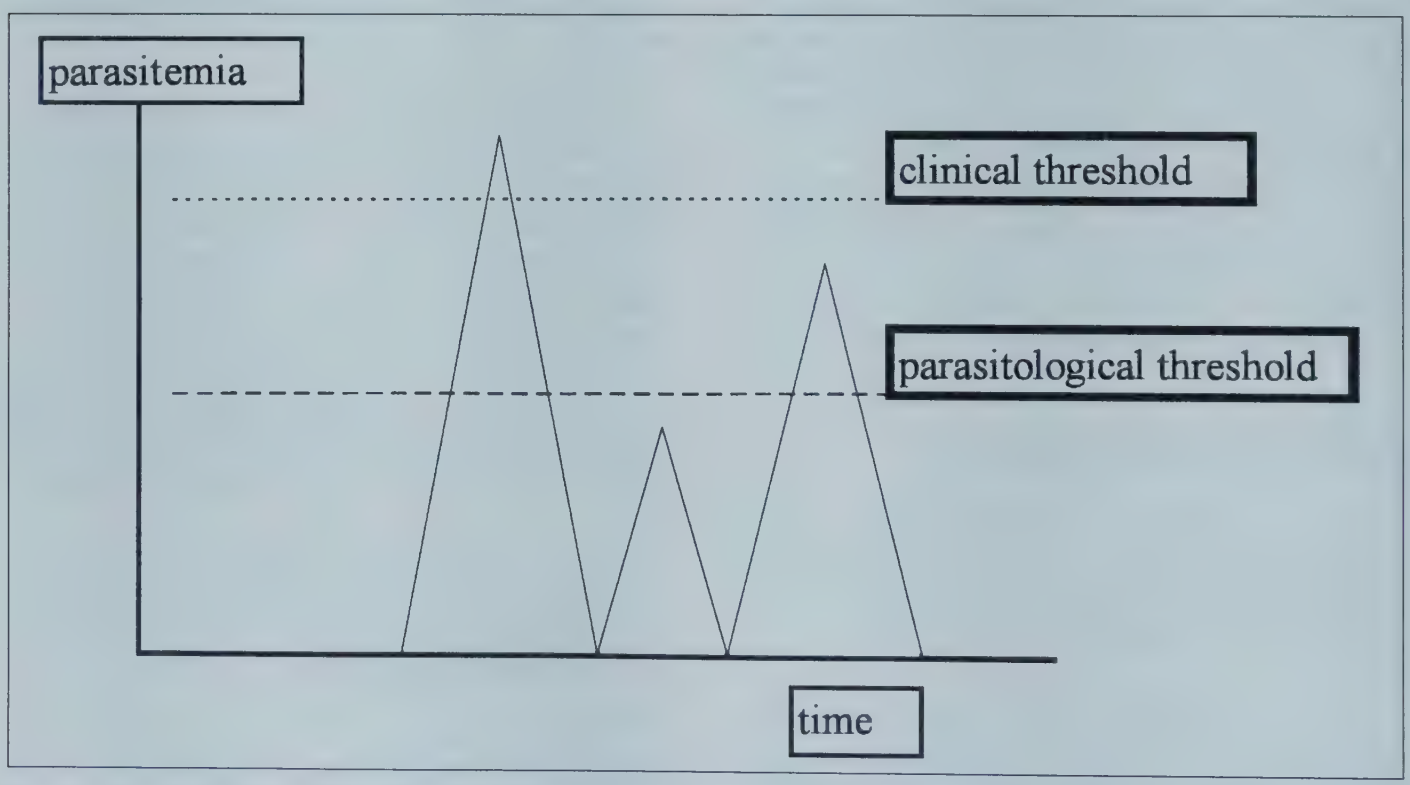

Fig. 1. Parasitological (lower) and clinical (upper) threshold. Some spikes of parasitemia cause clinical symptoms (the first), some are detectable but do not cause symptoms (the last) and some are not detectable by routine laboratory techniques (the middle).

able to malaria disease is the *clinical threshold*. It depends on the species and the strain of the parasite, and on the immunity of the patient. The number of infective bites, or the endemicity will play a role through the effect on the immunity. The drugs already taken and the relative resistance of the parasite against the drug have no influence. A "universal" clinical threshold does not exist: it will differ quite a lot between small children in hyperendemic ("malaria stable") areas, adults in the same regions, people in hypo-endemic ("malaria unstable") regions, displaced people, migrants, travellers and expatriate residents. In this respect, it is important to emphasise that immunity may fade temporarily in pregnancy, and temporarily or permanently in semi-immune people leaving the endemic region.

In holo-endemic regions, the clinical threshold is mostly several orders of magnitude higher than the parasitological threshold.

In travel medicine however, it is extremely rare to find parasites in the blood of an otherwise healthy traveller (that would mean that the clinical threshold would be higher than the parasitological threshold, as is the case in endemic regions). For *P. falciparum* infections the clinical threshold is mostly equal to the parasitological threshold. For *P. ovale* infections it may be lower than the parasitological threshold (cf. chapter "Clinical aspects of malaria"). Of course these statements hold true only for a highly skilled laboratory, where (in suspected cases) parasites are sought for at least 15 minutes in a thick film.

There are similar threshold examples in medicine, although there are some fundamental differences. The interpretation of the presence of microbes in the culture of midstream urine e.g. also requires the notion of a "clinical threshold" (more than 100.000 colonies per millilitre). The difference is that in this case an unequivocal indicator of morbidity is generally accepted: the presence of microbes in urine obtained by sterile puncture of the bladder. All tests will be evaluated against this "gold standard". In contrast to this, in malaria the clinical threshold has to be deduced from statistical analysis.

Test-treatment threshold

The former two thresholds should not be confused with the *test-treatment threshold*, which defines the level of evidence or disease probability at which one decides to forgo further testing and to start treatment. This decision can be made on a personal basis, in a clinical situation, or on a population basis, in the form of algorithms or strategies.

The determination of the test-treatment threshold depends on the benefit of the treatment, the risk and the cost of the treatment, the characteristics and the risk of additional tests (Pauker, 1980)[1] (figure 2).

For any disease,

- we do not need to be so certain of the diagnosis if it is a life-threatening disease, with a highly beneficial treatment. We would easily agree to treat some patients who do not have the disease. An example is meningitis caused by meningococci: fever, nuchal rigidity and petechiae are sufficient to start treatment immediately, without lumbar puncture if this can not be performed within minutes.
- we must be more certain of the diagnosis if the treatment is dangerous, or expensive. Example: African trypanosomiasis. Except for some rare cases, a parasitological diagnosis in blood, lymph nodes or spinal fluid is required before treatment is started.
- we should test further, and hence obtain a higher level of certainty if the next test has few false negatives. If the next test could miss a lot of cases however, we would prefer to treat immediately, without ordering the test. Example: if ultrasound is available, we should order it before starting treatment for amoebic abscess.
- we should treat at a lower level of certainty, and not order a test if it is expensive or dangerous (especially if the sensitivity or specificity are not very high). Example: X-ray of the chest for bronchitis.

For malaria,

- it is a life-threatening disease, with a highly beneficial treatment. It is acceptable to treat some patients who do not have the disease.

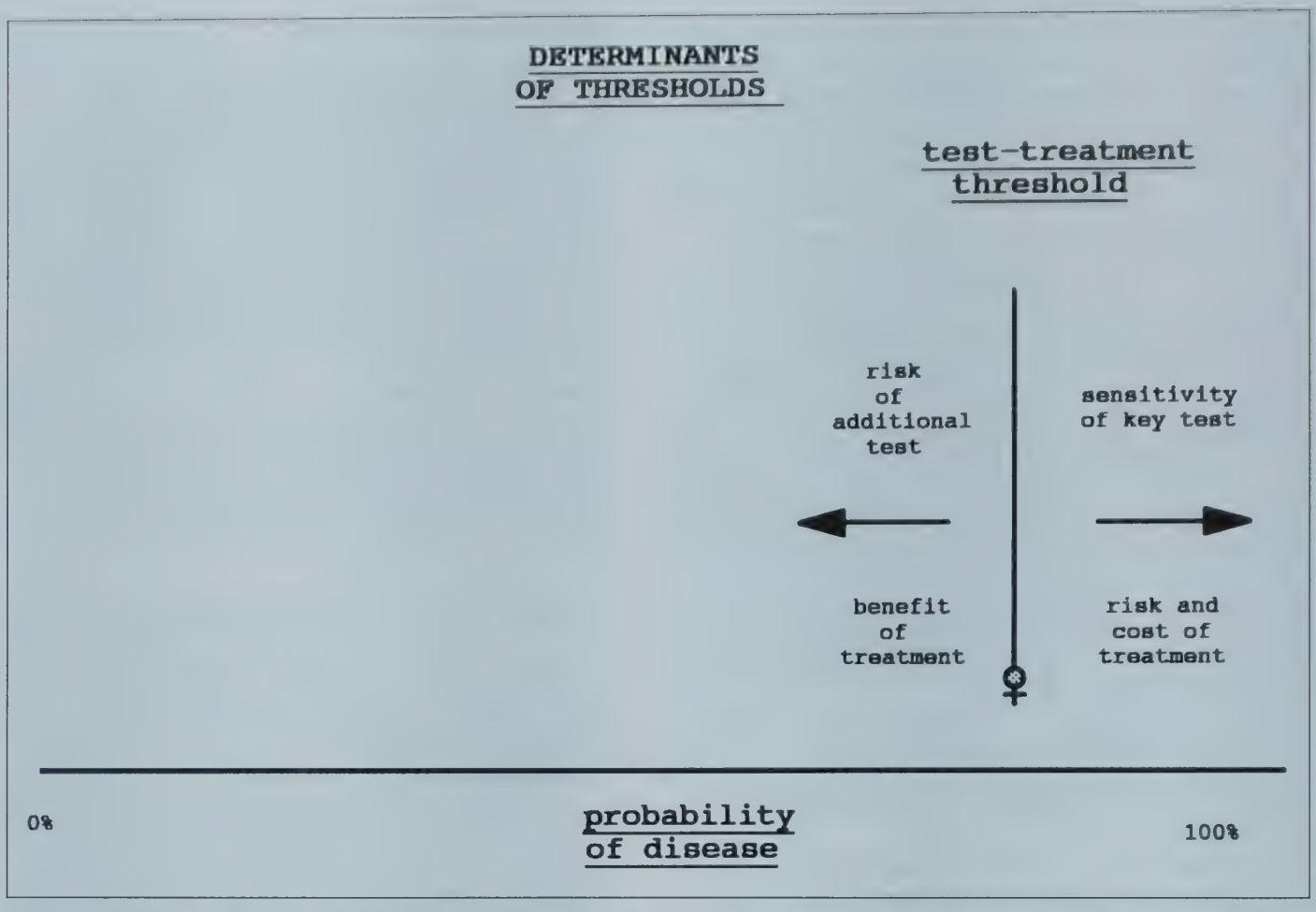

Fig. 2. Representation of the most important factors that influence the test-treatment threshold. The threshold for treating without further testing is shifted to the right in case of expensive or dangerous treatment and in case of a test that has few false negatives (highly sensitive). It is shifted to the left if further testing confers some risks or if the treatment is highly beneficial.

- the treatment is not dangerous, or expensive. This lowers our threshold.
- the thick film has few false negatives. It will miss almost no cases. This is an argument for doing the test, and treating at a higher level of certainty.
- the thick film is not dangerous, but it is expensive in field conditions. This brings the threshold down.

How to determine the clinical threshold?

For different settings, one could define a clinical threshold as a level of parasitemia above which the likelihood that the symptoms are caused by malaria is higher than the likelihood that the symptoms are provoked by another disease.

In recent years, a lot of work has been done in this field. Since there is no unequivocal specific indicator of morbidity, no gold standard for "malaria disease", one has to rely on statistical methods, which try to distinguish between fever attributable to malaria and other fevers. The best method is the "attributable fraction" that calculates the number of fevers due to malaria for different levels of parasitemia (Smith, 1994)[2].

Attributable fraction

Taking all cases of fever, the proportion due to malaria is the attributable fraction (AF). In patients who present with fever and whose thick film is positive for malaria parasites, only some are due to malaria, the remainder of the fevers being caused by other diseases. In patients with fever but with a negative smear, it is unlikely that the fever is caused by malaria (if not a *P. ovale* infection). So we presume that there is no malaria attributable disease in this group (figure 3).

How do we calculate the malaria attributable disease? First, let us assume that

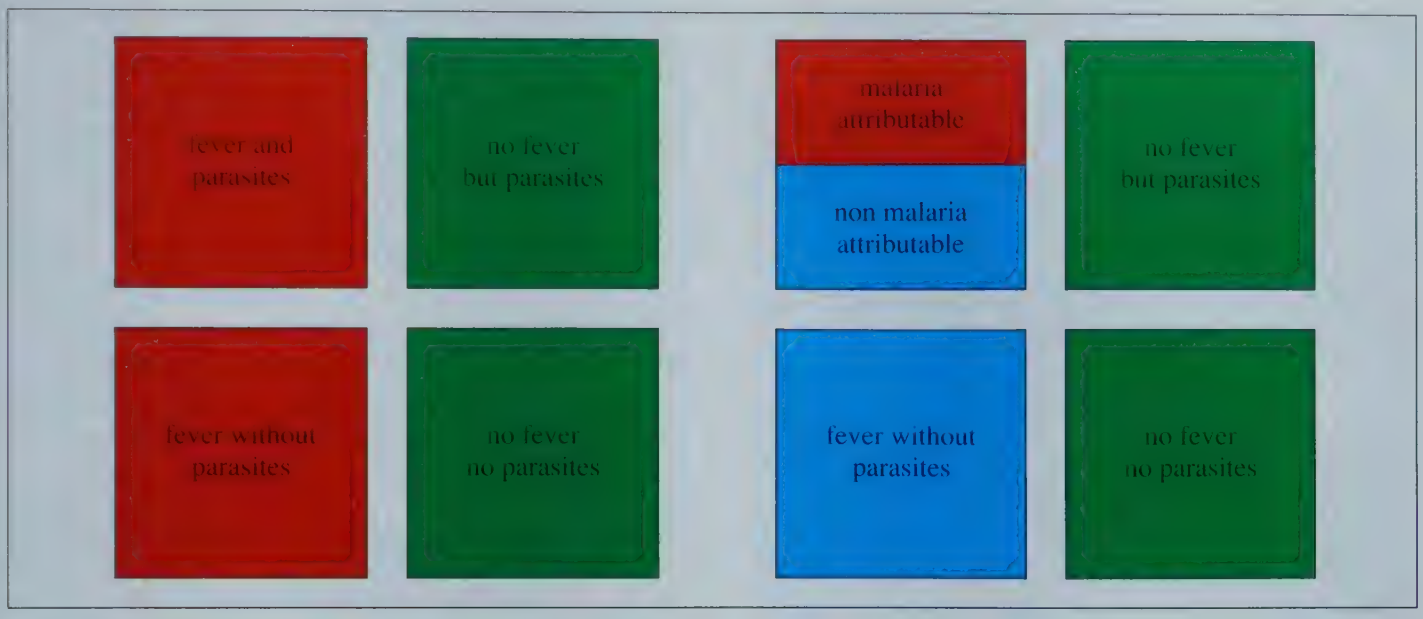

Fig. 3. Attributable fraction. On the left we see the four groups of patients, or categories of combinations of fever and parasites. On the right we see that only some of the fevers with parasites are malaria attributable. Fevers without parasitemia are not malaria attributable.

the proportion of febrile children amongst parasitemic children is called f_p, and the proportion of febrile children amongst aparasitemiac children is called f_{p0}.

Among the children with parasites and fever, the proportion of fevers due to malaria, is (*):

$$(f_p - f_{p0})/f_p$$

Further, if we call "p" the proportion of cases of fever with parasites, then the fevers due to malaria in all children with fever, or the attributable fraction (**) is:

$$AF = p(f_p - f_{p0})/f_p$$

Let us have a look at an example, drawn from a population study in children in the Gambia (Alonso, 1993)[3] (Table 1).

Intuitively, we would state that the presence of parasites is suggestive of malaria disease: more parasitemic children have fever (41/127, 32%) than aparasitemic children (33/280, 11%).

These numbers give f_p and f_{p0}:

$$f_p = 0.32 \text{ or } 32\%$$
$$f_{p0} = 0.11 \text{ or } 11\%.$$

Among the children with parasites and fever, the proportion of fevers due to malaria $(f_p - f_{p0})/f_p$ is:

$$(0.32 - 0.11)/0.32 = 0.63.$$

In normal language this means that 63% of children with fever and parasites are presumed to be ill with malaria, in the other 27% the fever is probably caused by another disease.

Among cases with fever, 41/74 or 0.55 are parasitemic, thus

$$p = 0.55.$$

The overall probability that the fever is caused by malaria (or the attributable fraction) will be:

$$AF = 0.55 \times 0.63 = 0.35$$

In normal language, this means that only 35 percent of all fevers are probably due to malaria.

	Fever	No fever	Total
Any parasite	41	86	127
No parasite	33	247	280
Total	74	333	407

Table 1. Presence of fever depending on parasitemia in a population study.

(*) This proportion is referred to as attributable risk.
(**) There is a lot of confusion about definitions: for some authors, the attributable in parasitemic patients fraction is the proportion of fevers due to malaria, $(f_p - f_{p0})/f$.

Attributable fraction for different levels of parasitemia

Let us look at another study of cases with and without fever, and with and without parasitemia seen in a setting in Mali (Rougemont, 1991)[4] (Table 2). This is a study starting from patients with fever, for whom an equal number of adequate controls were subsequently found.

It is intuitively clear that of all cases with fever, some will have other diseases than malaria, since controls also have parasitemias that may rise as high as > 100 000 per microlitre. On the other hand, it is intuitively clear that from 10 000 parasites on, people are more likely to be suffering from malaria. Finally, it is strange that we see more low parasitemia in controls without fever than in cases!

The AF can be calculated for different levels of parasitemia. The method faces a pitfall, also made clear in Table 2: in a hyperendemic region, a smaller number of children with low parasitemias are febrile than children without parasites. Patients with non malaria-attributable fever have a lower parasite density because fever or other physiopathological mechanisms re-

Parasite density	Cases (fever)	Controls (no fever)
>100000	43	7
25000-99999	61	15
10000-24999	19	10
1-9999	40	67
No parasites	122	186
Total	285	285

Table 2. Parasite density (in parasites/microlitre) and probability of fever in Mali.

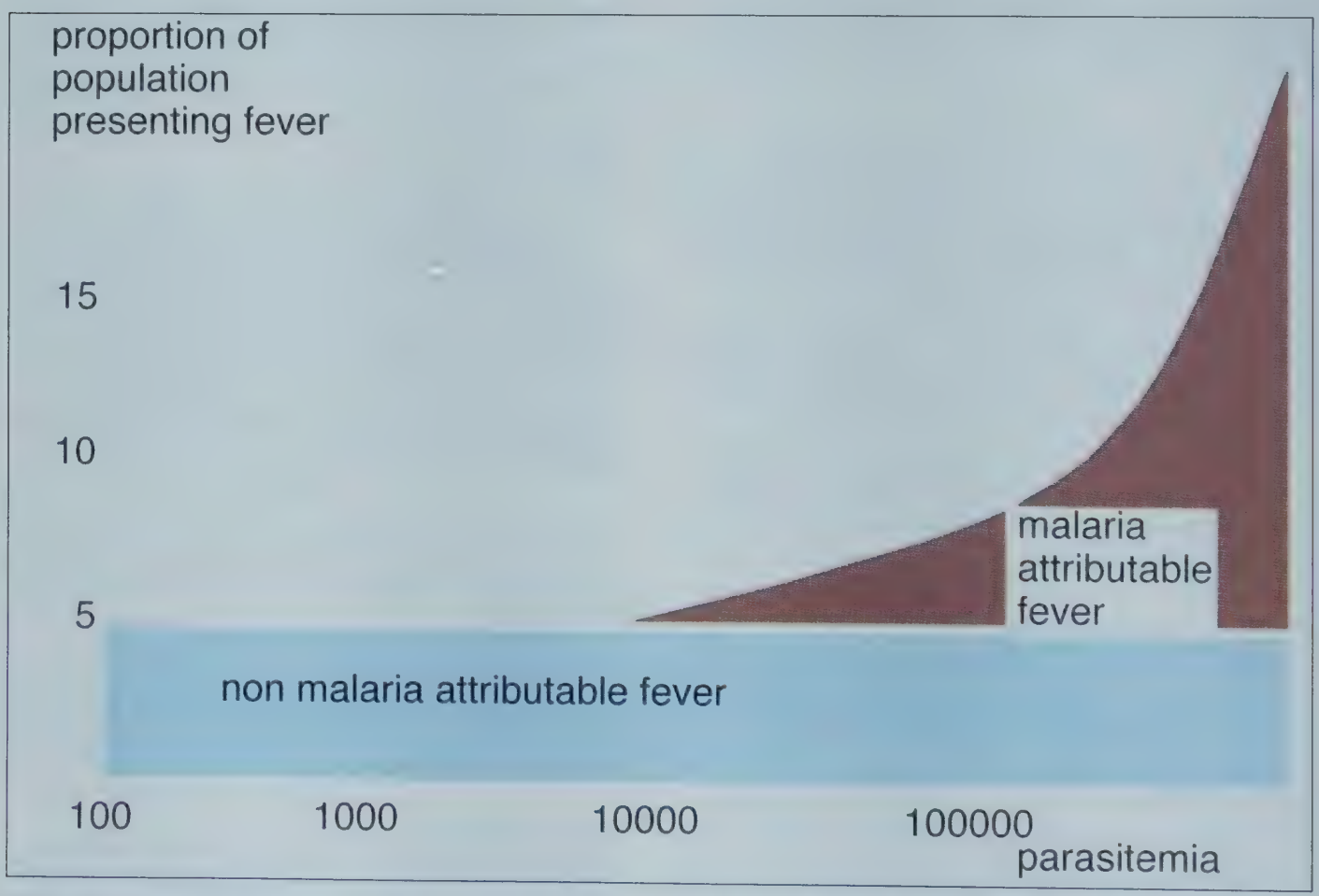

Fig. 4. Attributable fraction as a function of parasitemia level. From a certain level of parasitemia on, malaria attributable fevers appear, and become even predominant. Background non-malaria attributable fevers persist over the whole range of parasitemia [adapted from Smith (Smith, 1994)][2].

duce the parasite density. Mathematical corrections allow for a normalised model (figure 4) (Smith, 1994)[2].

The proportion of patients presenting with fever is shown for a range of parasitemias. From a certain point on, malaria enters the scene, once the attributable fraction exceeds 0.

In holoendemic areas, attributable fraction studies often put the mean clinical threshold at 10.000 parasites per microlitre (Baudon, 1988; Genton, 1994)[5, 6]. In meso-endemic or oligo-endemic regions this can be lower: e.g. in Thailand it was 1460/µL for *P. falciparum* and 181/µL for *P. vivax* (Luxemburger, 1996)[7].

The question whether the risk of malaria morbidity increases with age for any parasite density studied, in other words if the clinical threshold lowers with age, has not yet been answered. In recent studies, this thesis has been challenged (Petersen, 1991)[8]. There is certainly a decrease in mean body temperature with age for a given level of parasitemia, but for adults in a holoendemic region, there is no attributable morbidity at all (Smith, 1995)[9]! This corresponds with the finding in another study, that it was impossible to define a clinical case of malaria for adults (Petersen, 1991)[8].

Diagnostic pitfalls

In hyperendemic areas, we could incorporate the threshold logic. This should be done with caution, since there are some pitfalls:

- Combining a positive slide with fever, whatever the parasitemia, is not absolute proof of the diagnosis "clinical malaria". It might be that the parasites are not the cause of the fever, that an innocent viral disease or another condition is in fact the diagnosis. Combining a positive slide with fever for the diagnosis of malaria can therefore be dangerous. If the diagnosis of malaria is accepted, and if further clinical history taking and physical examination is careless, one could miss the diagnosis of pneumonia, infectious diarrhoea, meningitis...
- For a high parasitemia, statistically it will be more probable that malaria is involved, but it is not an absolute proof, since high parasitemias can be found in "healthy" children.
- Not to take into account a positive thick film in a patient might be dangerous too: even if he presents symptoms or signs that suggest another diagnosis such as pneumonia or dysentery, the symptoms could in fact be due to malaria, or he could have two diseases at the same time.

It is difficult to explain these warnings to health care workers in the field or to nurse practitioners, and even to medical doctors not trained in decision analysis. This is probably the reason why the results of so many malaria laboratory tests are not taken into account, even if they are available. Too often also, these results arrive too late to be useful in clinical decisions.

It is time to comment on the three cases we presented in the beginning of this chapter.

... One day in Zaire in 1976, one of us was called to the daughter of a colleague, who had fever and a convulsion. She was on daily chloroquine malaria prophylaxis...

For some cases, it has been shown in post-mortem slides that malaria was the cause of death, despite a negative thick film (Wolf-Gould, 1992)[10]. Most authors believe that this is the exception, rather than the rule (White, 1992)[11]. Normally, no fever is attributed to malaria if the thick film is negative (with the exception of *P. ovale* infections).

Could the thick film have been negative because of the chloroquine prophylaxis? The assumption that antimalarials bring parasitemia down under the detection threshold, and provoke a false negative slide is not correct: if patients still have fever, the thick film will be positive. Neither the intake of antimalarials, nor chloroquine resistance have an influence on the clinical threshold (which is – for *P. falciparum* – always equal to or higher than the parasitological threshold).

... A second case in the same region was a 32-year-old local chief, who presented one evening in 1984 with abnormal be-

haviour and fever of 38.5°C. A thick film showed scanty parasites of P. falciparum...

Was this fever and this abnormal behaviour attributable to the scanty parasites they found in the thick film? It is improbable. Petersen did not find malaria attributable disease in adults in a holo-endemic situation (AF = 0). Moreover, the low level of parasitemia makes a relationship still less probable.

... A third case is a 10-year-old child in Guinea-Conakry, that was seen with fever at the health centre. Chloroquine was given, but after three days....the thick film was scanty positive... the diagnosis of typhoid fever was made on clinical grounds and chloramphenicol was given...an ultrasound showed a liver abscess, probably amoebic.

This case shows that some fevers with positive slides are due to other causes, in this case an amoebic liver abscess. It was not very intelligent to go on with chloroquine the third day: if a patient is referred after two days, chloroquine resistance should be suspected, and a second line drug should be instituted. The presence of parasites after three days of treatment indeed suggests chloroquine resistance, even if this case was probably not malaria attributed. Furthermore, the result of the thick film should be obtained immediately, not the following day, in order to adjust decisions immediately.

Presumptive diagnosis versus confirmed diagnosis

Presumptive diagnosis

Introduction

In malaria endemic countries economic and logistic reasons force entire populations to rely on clinical diagnosis. This is not the only reason for presumptive diagnosis: when we take the difference between parasitological and clinical threshold, and the pitfalls of interpretation into account, the often heard statement *"it is mandatory to obtain the laboratory confirmation of the presence of malaria parasites in the patients body, whenever possible"* becomes debatable. If one cannot correctly interpret the presence of parasites in the blood of the patient, parasitological diagnosis of malaria is not useful, and might be dangerous. Even if all laboratory tools are available, in most hyperendemic regions the laboratory confirmation of malaria is not useful at all levels. Presumptive diagnosis is thus not only a strategy of poverty, but can be defended scientifically.

Rationale

What defines a presumptive diagnosis is the decision to forgo further testing and to start treatment. As we explained earlier, this decision is made at the *test-treatment threshold*, the required level of certainty or probability that the patient has malaria. As was also explained, this involves the probability that the patient has malaria-disease and not only malaria infection.

The determination of the test-treatment threshold depends on several factors: the benefit of the treatment, the risk and the cost of the treatment, and the characteristics and the risk of an additional test (figure 2).

As already explained, the benefit of treatment for malaria is high, and withholding a treatment from someone really suffering from malaria can have serious consequences. The risk of withholding treatment is dependent on the false negative rate of the case definition. If the case definition is based on fever, patients with malaria-attributable symptoms but without fever will not be treated. Some of them may have severe malaria.

The risk for serious side-effects of most first-line malaria treatments is low: even for sulfadoxine-pyrimethamine this risk is considered to be between 1 in 10^4 and 1 in 10^7 (Stürchler, 1995)[12]. Except for some countries with high level multiresistance, the cost of first-line treatment is also low.

The risk of additional testing is non-existent. The cost, however, is considerable, since microscopes have to be purchased, an unaffordable expense in most rural settings.

With all these elements an estimate can be made as of how many patients we accept to treat unnecessarily. With the attrib-

utable fraction methodology, we can determine which clinical case definition is acceptable for the test-treatment threshold already set.

If in the example of the Gambia we determine a clinical case definition as "fever", we will treat 65% of fevers that are not malaria attributable with chloroquine. Is this acceptable? When the resistance to chloroquine is high and the first line drug is sulfadoxine-pyrimethamine, one might prefer a higher threshold.

Algorithms

Substantial efforts have been made to refine presumptive diagnosis. A comparison between the clinical threshold (defined by the attributable fraction) and clinical symptoms and signs can result in a better case-definition. However, we should not forget that relying on concomitant symptoms and signs will result in a higher specificity (fewer false positives), with a loss in sensitivity (more false negatives, some patients with malaria disease will not be treated) (Rougemont, 1991, Baudon, 1988, Redd, 1996, Genton, 1994, Schellenberg, 1994, Van den Ende, 1996)[4, 5, 13, 6, 14, 15]. Some authors suggest including measured temperature, nailbed pallor, and splenomegaly in the case definition (Redd, 1996)[13]; others suggest more complicated algorithms, at the cost of more false negatives (Rougemont, 1991)[4]. Still others find that no algorithms can do better than the health workers' intuitive estimation (Bassett, 1991)[16].

Generally, fever is considered to be the key symptom of malaria, and a presumptive diagnosis of malaria is made starting from fever. However, in a holoendemic setting in Tanzania, Smith *et al.* found that for infants, a large proportion (66.5%) of malaria-attributable disease presents without fever. For older children, this is less than 40%, but is still considerable.

In holoendemic settings, fever is indeed a frequent complaint and a reason for consultation. Moreover, it might be that patients have such a high likelihood of malaria disease that further laboratory testing is senseless. In a holoendemic setting, the definition of malaria as "fever plus parasites" can be almost as non-specific as fever alone (Schellenberg, 1994)[14]. Of all children consulting for any illness in two settings in Malawi, 983/1124 had a history of fever, 672/1124 had parasites, and 285 had a parasitemia > 10.000 per microlitre (malaria attributable). Of those with fever, 624/983 had parasites and 272/983 had a parasitemia > 10.000 per microlitre (Redd, 1996)[13]. In Papua New Guinea, 32% of children < 10 years with fever had more than 10.000 parasites per microlitre (Genton, 1994)[6]. In Burkina Faso, more than half of fever cases (> 38°) in children 5-14 years had more than 10.000 parasites per microlitre (Coulibaly, 1991)[17]. In the Gambia a community survey showed that 44% of febrile children had malaria attributable disease (Schellenberg, 1994)[14]. However, in the already mentioned study in Tanzania by Smith *et al.*, lower figures of malaria attributable disease where found: for all episodes of whatever reported illness, 9.8% was malaria attributable in infants, 1.3% in children 1-4 years-old, 0.6% in children 5-9 years-old. No malaria attributable disease was found in adolescents and adults (Smith, 1995)[9].

The assumption that fever equals malaria may be wrong and dangerous in a hypo-endemic setting or during the low transmission period. In Zimbabwe, only 28% of patients with "clinical malaria" had any parasites in their blood (Basset, 1991)[16]. Only 10 of 277 patients with fever had a real malaria attack during the dry season in Niger, whereas the personnel made the diagnosis of "clinical malaria" in 270 of them (Olivar, 1991)[18]! If the probability of malaria drops below a certain level, presumptive diagnosis has no sense, and should be replaced by a parasitological diagnosis.

As a conclusion for algorithms we could state that:

"fever does not equal malaria"

but, after application of decision analysis

"fever equals malaria treatment in holoendemic areas, in the first line"

Confirmed diagnosis: some additional remarks

Holo-endemic areas

If we incorporate the threshold logic in holo-endemic areas, we should be aware

of the fact that a thick film showing fewer parasites than the presumed clinical threshold does not exclude malaria but that it makes malaria disease as the cause of the patient's current clinical condition less probable. A thick film above the threshold is also not absolute, it does not give a verdict, but it shifts the emphasis in the differential diagnostic process towards malaria, not excluding other diseases.

Offering laboratory facilities to health centres should always be combined with a training in interpretation of results. This is difficult especially for malaria, and it is rarely done.

Too often, presumptive diagnosis creeps into second and third line medicine. Since other differential diagnoses are much more frequent at this level, and since treatment is more complicated, the medical decision logic for presumptive diagnosis is absent, and it should be abandoned. A parasitological diagnosis at the hospital level is mandatory. Threshold logic is also useful here: negative slides and strongly positive slides will be of more interest than moderately positives.

Hypo or non-endemic regions

In hypoendemic regions the presence of parasites in the blood of healthy people is less frequent or rare. In these situations a confirmation by laboratory tests is strongly advocated, if economic and logistic constraints permit it (Olivar, 1991; Jonkman, 1995)[18, 19]. In non-endemic regions (travel medicine, migrants, displaced people...) the confirmation of suspicion is mandatory.

Clinicians in non-endemic countries need to be well aware of the possibility of imported malaria cases. Diagnosis in these cases may be difficult since the exposure to the infective bite may date back to a long time before clinical symptoms appear, especially in the case of *P.vivax*, *P. ovale* or *P. malariae* infections. The accurate geographical history (unde venis?) should be a routine in every history taking of cases of fever even in the malaria-free world. Finally, it has to be stressed once more that *P. falciparum* infections in non immune subjects (travellers from non endemic western countries, migrants visiting their home country, ...) may be extremely severe, with high case-fatality rates if correct diagnosis and treatment are not carried out promptly.

It is clear that in imported malaria, in low endemicity settings, in research and epidemiology, the more sensitive tools like PCR, antigen detection and the like can be of value, but rarely in clinical medicine in holoendemic regions. In most malarious regions, we should look for *less sensitive* diagnostic tools. In a holoendemic setting, this could be solved by making a diagnosis with a thin smear instead of a thick film. The thin smear is less sensitive, but it is more specific and the technique is easy and the drying time much shorter.

Additional laboratory tests

For a classical malaria attack, a simple panel of haematological examinations (haemoglobin level, red blood cell count, hematocrit, white blood cell count, thrombocytes) are recommended.

The finding of marked anaemia, contrary to what was stated in older handbooks, is not a feature of classical malaria: marked anaemia is never the result of a simple infection, it is the hallmark of (an incipient) severe malaria, in its acute or subacute form.

The thrombocyte count is rarely normal in malaria infection by all species, it often falls below 50 x 10^9/lit in falciparum malaria. In imported malaria, the positive predictive value of the triad fever, history of a recent stay in endemic areas and thrombocytopenia below 100 x 10^9/lit has been found to be as high as 97% (Castelli, 1995). It is of course clear that a laboratory that reaches the level of sophistication of a thrombocyte count, should be able to interpret a thick film correctly !

No specific diagnostic indications are given by the white blood cell count, since the result may be normal, low or high. In severe malaria however, a high leucocyte count indicates a poor prognosis (see chapter on "Clinical aspects of malaria").

Haptoglobin level, total cholesterol and HDL cholesterol are all very low in malaria, and could be of help in a situation where malaria should be ruled out and a reliable parasitological diagnosis can not be per-

formed. However, we refer to the same remark as for the thrombocyte count.

As pointed out in chapter on "Clinical aspects of malaria", other laboratory tests can be of help in the diagnosis and the management of severe and complicated malaria: glycaemia, liver and kidney function tests, electrolytes, blood gas analysis, serum lactate, fibrin split products. These tests have no place in uncomplicated malaria: as said before, finding one of these tests to be significantly abnormal shifts the diagnosis from classical to severe malaria, with important consequences for treatment and management.

The utility of lumbar puncture in cerebral malaria has been questioned. The distinction between cerebral malaria and meningitis can be difficult, as cerebral malaria can present with nuchal rigidity, and meningitis can present without. Wright *et al.* found 7.2% of cerebral malaria with, and 36% of meningitis without nuchal rigidity in comatose Liberian children. They strongly advocate a lumbar puncture for every comatose child in malarious regions, since the clinical distinction between both diseases is not reliable (Wright, 1993)[20].

Differential diagnosis

The extremely wide spectrum of clinical symptoms and signs occurring during malaria disease is covered in chapter (Clinical aspects of malaria), and exceeds the purpose of this chapter. A brief and non exhaustive list of possible differential diagnoses of falciparum malaria key symptoms and signs is nevertheless given in Table 3.

Presenting symptom/sign	Notes	Differential diagnosis
Fever	Often irregular or continuous at onset	Influenza, dengue, brucellosis, sandfly fever, acute respiratory infections, viral hemorragic fevers, tuberculosis, enteric fever, trypanosomiasis, visceral leishmaniasis, rickettsial fever, relapsing fever
Coma	Usually (not always!) high parasitaemia is present	Meningitis, encephalitis, typhoid fever, cerebrovascular accidents, trauma, alcoholism, hypoglycaemia
Jaundice	More common in severe malaria for which it is a defining criteria	Infectious hepatitis, leptospirosis, disease of the biliary tract
Hepatomegaly	Usually tender in the acute phase	Amoebic liver abscess, beri-beri, brucellosis, leptospirosis, relapsing fever, viral hepatitis, thalassaemia
Splenomegaly	Common in all types of malaria and usually tender in the acute phase	Brucellosis, relapsing fever, trypanosomiasis, typhoid fever, leptospirosis, thalassaemia
Renal failure		G6PD deficiency, sickle cell disease, snake venoms, leptospirosis, traditional herbal remedies, heat stroke
Gastrointestinal symptoms	All kinds of gastrointestinal symptoms (ranging from vomiting to choleraic diarrhea)	All types of dysentery, cholera, gall bladder disease, acute pancreatitis, acute appendicitis

Table 3. Differential diagnosis of the presenting clinical symptoms/signs in malaria infection (adapted from Gilles, 1988).

Conclusion

The diagnosis of malaria disease differs substantially in holo- and hypoendemic regions. Where in hypoendemic regions it equals parasitological diagnosis, in holoendemic regions the diagnosis depends on the clinical threshold, which can be found on a population basis by defining the attributable fraction for different levels of parasitemia.

A presumptive diagnosis of malaria is not a strategy of poverty: it has formal scientific grounds, that rely on the theory of the test-treatment threshold. It should be restricted to holo-endemic malarious regions, and to the first line. Parasitological diagnosis remains mandatory in district hospitals and reference hospitals.

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ANTIMALARIAL DRUGS

W. H. Wernsdorfer

History of antimalarial drugs

Paleological evidence suggests that man became acquainted with malaria at the dawn of humanity in tropical Africa[1]. While the plasmodial infections seemed to have had a microfocal distribution at that time, wider populations became involved after settling down and adopting agricultural existence. By the end of the Neolithic period it seems that all major centres of human development were affected by malaria, with the possible exception of the New World. Until the discovery of *Plasmodium falciparum* by Laveran in 1880[2] the cause of malarial fevers remained shrouded although the names of "malaria" and "paludisme" point to the empirical association between the fevers and swamps.

Attempts of treating malarial fevers must be as old as man's association with plasmodia, but historical evidence indicates that the first truly effective medication, in the form of the bark of Cinchona trees, came into recorded use during the first half of the 17th century[1]. Claims of the antimalarial efficacy of preparations of *Artemisia annua*, an old Chinese herbal remedy for various ailments[3], cannot be substantiated since the artemisinin content of the infusions could not have reached a therapeutic level.

While the romantic tale of the discovery of the antimalarial properties of Peruvian bark has been relegated to fiction it is a fact that the bark of the "fever tree" (*Cinchona* spp.) was brought to southern Europe during the first half of the 17th century and successfully used there against malarial fevers[1]. Within a few years Peruvian bark became the universal remedy against malaria and other febrile conditions.

Still, the use of *Cinchona* bark was beset by uncertainties, from varying alkaloid contents to deterioration of biological activity due to storage. These constraints ended in 1820 with the extraction of the pure *Cinchona* main alkaloids by Pelletier and Caventou[4]. Shortly after this event the use of Peruvian bark gave place to that of the pure alkaloids. Meanwhile *Cinchona* plantations have come up also outside the American Continent, especially in tropical Asia, nearer to the main consumers. At this time the precise role of the four *Cinchona* main alkaloids was not yet known, but in the late 1860s, well before the discovery of the malaria parasites, a large clinical trial under the auspices of the Madras *Cinchona* Board showed that quinine , quinidine, cinchonine and cinchonidine all possess antimalarial activity[5]. However, quinine became the main anti malarial agent on account of its abundance in the total extract (~ 90%) and due to the cardiological needs for quinidine which left practically nothing for other uses.

The early search for alternative, synthetic antimalarials was motivated by toxico-

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logical reasons as quinine is associated with a typical assortment of side effects well known under the term of cinchonism. As early as 1891 methylene blue was recognized to possess slight antimalarial activity[6], a discovery that generated the development of a concept of drug receptors in malaria parasites which maintained its actuality and usefulness for more than hundred years.

However, the main impetus to the development of synthetic antimalarials came from problems in the supply of quinine during the first World War when sea blockades and submarine warfare Interrupted the flow of the vital medicament to the war theatres in malarious areas[1]. Some 10 years after the war the first synthetic antimalarial, pamaquine, an 8-aminoquinoline, became available. As it has low blood schizontocidal activity it was no real replacement of quinine.

Further efforts yielded in 1932 mepacrin, an aminoacridine with blood schizontocidal properties, which could be used for treatment and prophylaxis. Although not free of side effects, it was far better tolerated than quinine. Meanwhile it was shown that pamaquine exerts radical curative activity in vivax malaria, a finding that lead to a wider exploration of 8-aminoquinolines culminating in the discovery of primaquine.

Simpler use and better tolerability were the motors of the search for new antimalarials during World War II and during the immediate post-war period. The efforts were successful in the discovery of chloroquine and amodiaquine, both 4-aminoquinolines with blood schizontocidal activity, and the antifols pyrimethamine and proguanil which were mainly used for prophylaxis.

Soon it became obvious that the large-scale prophylactic use of pyrimethamine was associated with rapid development of resistance, both in *P. falciparum* and *P. vivax*, but little attention was focused on this point since chloroquine had maintained its full therapeutic and prophylactic activity. The almost simultaneous occurrence of chloroquine resistance of *P. falciparum* in South America and on the Indochina Sub-Continent[7, 8] as well as the war in Vietnam re-stimulated chemotherapeutic research. From this time on, specific drug resistance of *P. falciparum* remained the strongest motive for the development of new antimalarials. Sulfonamide/pyrimethamine formulations were the first replacement drugs in areas with chloroquine-resistance, but increasing failure, especially in South America and on the Indochina Sub-Continent, necessitated new alternatives in the form of simultaneous use of quinine and tetracyclines, and later the deployment of mefloquine, a drug originating from the research programme of the Waiter Reed Army Institute of Research.

Resistance to quinine, mefloquine, and halofantrine in parts of the Indochina Sub-Continent has definitely accelerated the operational use of artemisinin derivatives, especially artesunate and artemether, which can currently be considered the last line of antimalarial therapy. This does not mean that the search for new antimalarials has ended. There are several interesting compounds deserving further development, but the situation is aggravated by reduced engagement of the pharmaceutical industry and the phasing out of research on antimalarials at institutions traditionally and successfully involved in this area.

Effect of antimalarial drugs (pharmacodynamics)

All species of the genus *Plasmodium* are host-bound throughout their life cycle. Sporogony, i.e. the part of the life cycle from gametogony until the production of viable sporozoites, is passed in the poikilothermal arthropod host. The development from the inoculation of viable sporozoites until the formation of viable gametocytes takes place in the homoeothermal vertebrate host as far as primate, rodent and avian plasmodia are concerned. In mammalian plasmodia the viable sporozoites invariably invade liver cells where they will undergo asexual schizogony ending with the liberation of merozoites which will invade erythrocytes. In some species, e.g. *P. vivax*, *P. ovale* and *P. cynomolgi* the sporozoites may transform into latent hypnozoites which will start pre-erythrocytic schizogony later and lead to relapses. After their interiorization into ery-

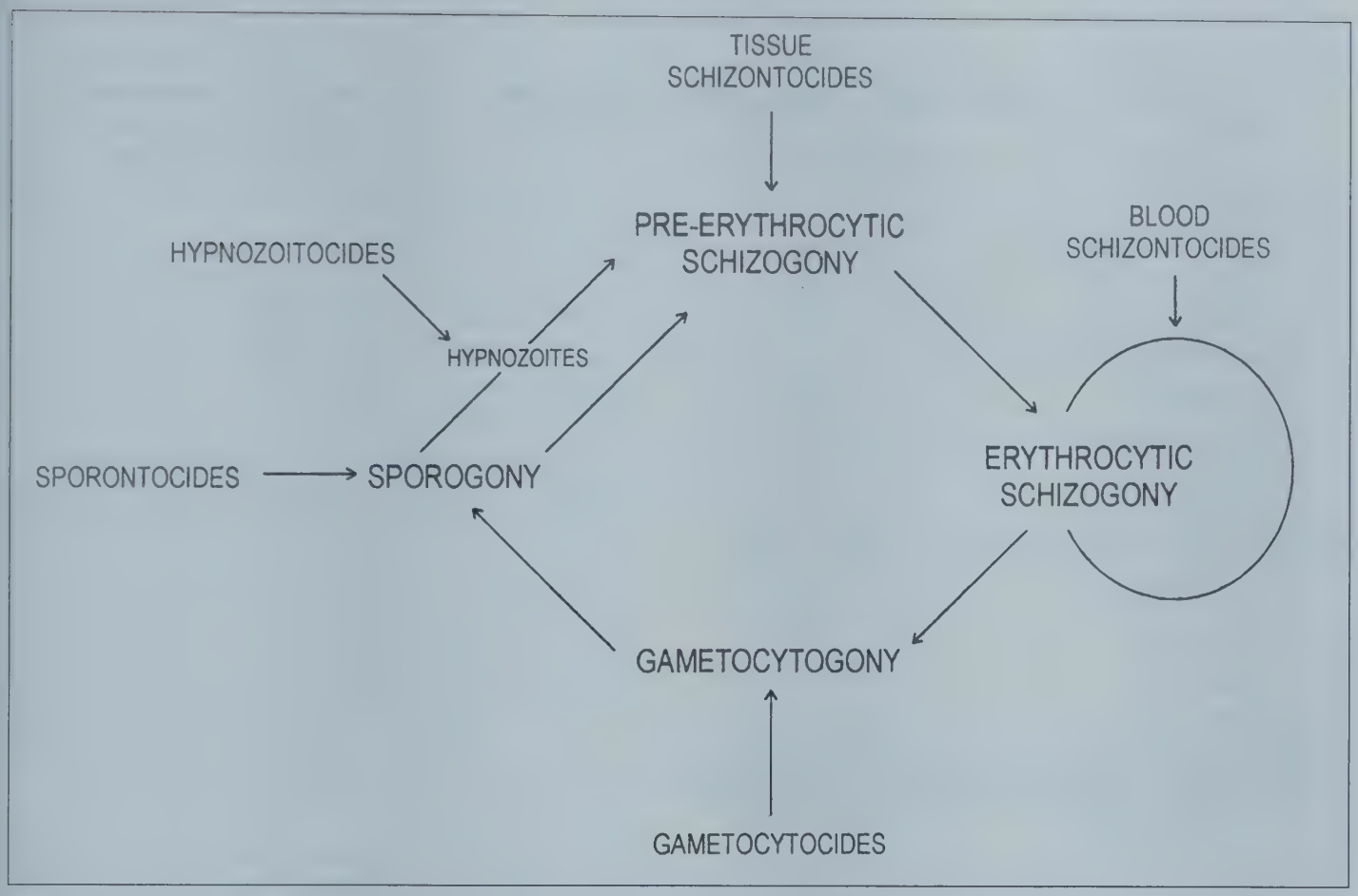

Fig. 1. Type of activity of antimalarial drugs in relation to the plasmodial life cycle.

throcytes, the merozoites will transform into trophozoites which will develop to preschizonts and schizonts ending with the production of erythrocytic merozoites. In due course these will escape from the host cell and infect other erythrocytes. This phase, or sub-cycle, is called asexual, erythrocytic schizogony. Some merozoites will give rise to the development of male and female gametocytes, i.e. the forms infective for the arthropod host.

This extraordinary variety of physiological conditions during the life cycle of the plasmodia is reflected in a rich variety of biochemical conditions and requirements. It is therefore understandable that the activity of specific antimalarial drugs may be directed against certain stages of the parasite's life cycle while other stages may suffer little or no harm.

In addition, the various species of *Plasmodium* show vast differences in their physiological behaviour and requirements. This pertains to the adaptation to specific arthropod vectors, the duration and abundance of pre-erythrocytic schizogony, the ability of producing hypnozoites, the duration and abundance of erythrocytic schizogony, the ability of merozoites to invade specific types of erythrocytes, the ability of inducing the sequestration of infected erythrocytes in small blood vessels, the duration of gametocytogony, and the viability of gametocytes.

Figure 1 shows the areas of interaction of drugs with parasites at specific parts of the life cycle. Thus, the following classes of drug activity can be differentiated.

Blood schizontocides

Drugs interfering with erythrocytic schizogony. Since this part of the parasite's life cycle is responsible for the clinical manifestations of malaria this is, therapeutically, the most important class of antimalarials. There may be major differences in the points of interaction, e.g. 4-aminoquinolines and 4-quinoline methanols will inhibit the development of trophozoites but show little effect once the division of chromatin has started. Antifols, however, will interact mainly at the

TARGET / DRUG	Sporozoites	Hypnozoites	Pre-Erythr. Schizonts	Asex. Blood Forms	Gametocytes *P. falciparum*
Chloroquine	–	–	–	++	–
Amodiaquine	–	–	–	++	–
Quinine	–	–	–	++	–
Mefloquine	–	–	–	++	–
Halofantrine	–	–	–	++	–
Primaquine	–	+	+	±	+
Pyrimethamine	–	–	?	+	–
Proguanil (*)	–	–	+	+	?
Sulfadoxine	–	–	–	±	–
Dapsone	–	–	–	±	–
Doxycycline	–	–	+	+	?
Tetracycline	–	–	+	+	?
Clindamycin	–	–	?	+	?
Artemisinin (**)	–	–	–	++	?

(*) Includes activity of main metabolite cycloguanil.
(**) Relates to artemisinin and active derivatives.

Table 1. Type of activity of various antimalarial drugs in the main human-pathogenic species of *Plasmodium*.

stage of chromatin division and show only moderate or no activity at the trophozoite stage.

Gametocytocides

Drugs acting against gametocytes at various stages of their development. This activity is often species-specific. Thus some drugs may be gametocytocidal in *P. vivax*, *P. ovale* and *P. malariae*, but show no effect against gametocytes of *P. falciparum*.

Sporontocides

Drugs directed against any parasite forms occurring in the arthropod host, from gametes to sporozoites.

Tissue schizontocides

Drugs inhibiting or killing pre-erythrocytic schizonts in the hepatocytes. This activity essentially relates to schizonts during active development as opposed to hypnozoites. This class of drugs was formerly called "primary tissue schizontocides".

Hypnozoitocides

Drugs effective against the "latent" hypnozoites. These are anti-relapse drugs. Formerly they were called "secondary tissue schizontocides".

One and the same drug may exert different types of activity, e.g. blood schizontocidal and gametocytocidal. Other drugs again may be gametocytocidal in some *Plasmodium* species, but not in others. Without going into details of the biochemical mode of action the rough classification of various drugs according to their activity against parasite forms occurring in man is given in Table 1 for the four main species of human-pathogenic malaria parasites i.e. *Plasmodium* (*Laverania*) *falciparum*, *Plasmodium* (*Plasmodium*) *vivax*, *Plasmodium* (*Plasmodium*) *ovale* and *Plasmodium* (*Plasmodium*) *malariae*.

Pharmacokinetics

When a drug is administered via the in-

travenous route it becomes 100% bioavailable. Moreover one can expect that the maximum drug concentration in the blood (C_{max}) will be reached at the end of the intravenous drug administration. In this case the time to maximum or "peak" concentration (t_{max} or t_p) will be identical with the time required for the intravenous drug administration. The drug is now in the blood or in the "First Compartment". With the blood it will reach practically the whole body where it may leave the blood to a lesser or greater extent, depending on the drug's affinities and the uptake in various tissues and organs. The "Volume of Distribution" (V_d) denotes this characteristic. There are drugs which mainly stay in the blood, e.g. quinine, showing a narrow or low V_d, while others, e.g. chloroquine, have an extremely wide distribution or high V_d.

A certain part of the drug may be bound to plasma proteins. The drug will be subsequently subject to metabolism and elimination and as a result the concentration of metabolites will rise and that of the parent drug decline, Metabolism takes place mostly in the liver, while elimination is mostly through urine and/or faeces. In the latter case there may be biliary excretion followed by partial re-absorption of the drug (entero-hepatic cycle). Finally, the concentrations of drug and metabolites will drop below detectable level. The speed with which this happens depends on the drug and on the subject. Plasma binding is often increased in acute febrile conditions due to enhanced binding in the presence of α1 acute stage glycoprotein. At the same time renal and hepatic function may be reduced by the underlying disease. This may lead to delayed metabolism and elimination. Thus the concentration-time curve may increase in comparison to healthy subjects. The integral of the concentration-time curve is called the "Area under the Curve" (AUC). The time point at which 1/2 AUC is reached is called the "Half-life" of the drug ($t_{1/2}$). There are antimalarials with a rather short $t_{1/2}$, e.g. quinine with 8-12 hours, and others with a very long $t_{1/2}$, e.g. mefloquine with ~ 20 days.

When an antimalarial drug is given by

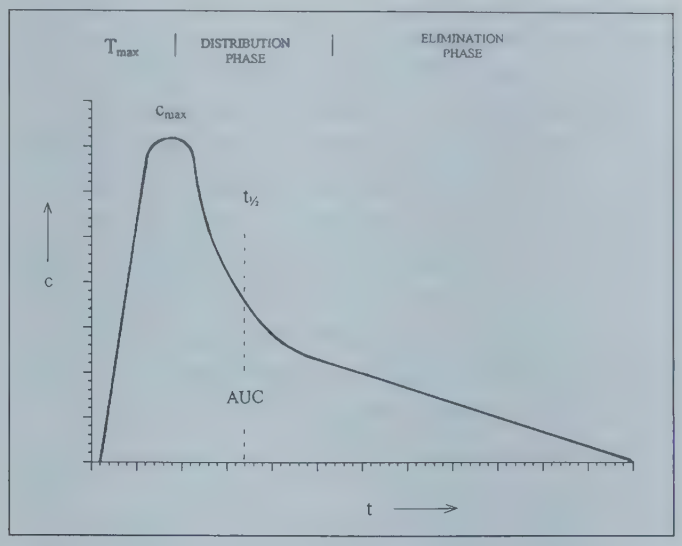

Fig. 2. Schematic concentration-time curve of a drug following oral administration.

the oral route it needs to be absorbed in order to reach the site of action. Absorption may take place in various parts of the gastro-intestinal tract and vary according to specific characteristics of the drug and the presence of food substances. Drugs which are mainly absorbed in the pylorus and duodenum will show a low t_{max}, while those mainly taken up in the ileum will show a relatively long t_{max}. After absorption in the gastro-intestinal tract the drug will have to pass the liver and may be there subject to early metabolic modification (first pass effect). From now on the drug will be in the circulating blood and undergo distribution, metabolism and elimination as mentioned above. A typical concentration-time curve after the oral administration of a drug is shown in figure 2.

A special case are drugs with little or no biological activity which are metabolically transformed into active compounds. Examples of such "pro-drugs" among the antimalarials are proguanil and chlorproguanil which, through the action of cytochrome P-450, are metabolized to the active compounds cycloguanil and chlorcycloguanil, respectively.

While the main pharmacodynamic effect of the drug should be directed against the malaria parasite it has to be realized that many antimalarials exert additional pharmacodynamic activity which may be useful in the treatment of conditions other than malaria (e.g. prevention of nocturnal

muscle cramps by quinine). However, most of the additional activity consists of undesirable, sometimes dangerous adverse effects, e.g. cardiovascular collapse after the intramuscular administration of chloroquine with doses in excess of 3.5 mg / kg body weight. In many cases cardiovascular side effects are concentration-dependent. It is therefore desirable to avoid sudden peak concentrations which are of little use from the antimalarial point of view and only carry the risk of severe adverse reactions. Thus quinine hydrochloride for emergency treatment is not given by (even slow) intravenous bolus injection, but only by rate-controlled intravenous infusion at a maximum speed of 5 mg/kg and hour for an initial duration of 2 hours, or 4 hours when a loading dose is indicated. This permits a rapid building up of the drug concentration level without the risk of severe adverse reactions.

The immediate goal of the treatment in severe or complicated malaria (*P. falciparum*) is the resolution of severity and complications. This is usually achieved by the parenteral administration of suitable antimalarial drug formulations and ancillary management according to the clinical condition[9]. After the successful management of such cases and in ordinary, non-severe malaria the achievement of radical cure, i.e. the elimination of the infection, will usually be desirable.

With drugs acting through an inhibition of the parasite's metabolic pathways radical cure will necessitate the maintenance of effective drug concentrations over a minimum time-span. The critical concentration level, often called MIC (minimum inhibitory concentration) or Cmin, is close to the EC-99 of the parasite population and the critical time span (tmin) comprises the time required for 3-4 cycles of erythrocytic schizogony[10]. This is shown in figure 3.

Antimalarial drugs differ in their pharmacokinetic characteristics. The dose regimens should therefore be adjusted in a way that guarantees the maintenance of drug levels above the Cmin for the required time span (tmin). With some drugs this will require only a single dose, e.g. sulfadoxine/pyrimethamine. With others 8-hourly dosing for 7 days will be needed, e.g. quinine. With others yet, an adequate

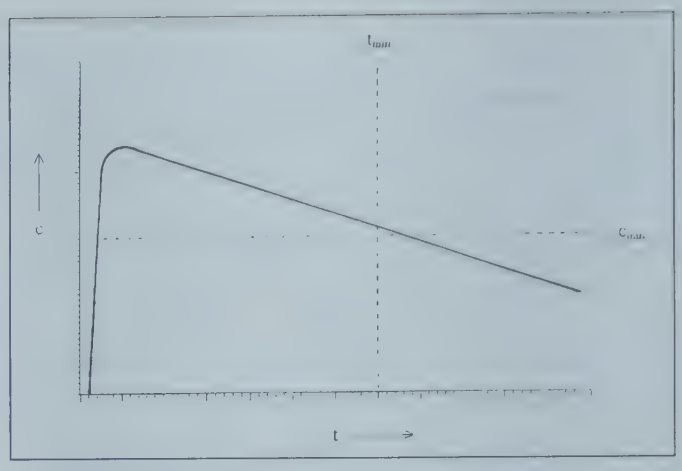

Fig. 3. Pharmacokinetic conditions for the complete elimination of asexual blood forms of plasmodia when using enzyme inhibitors.

dose profile could be, theoretically, obtained with a single dose, but split doses or dosing over several days will be required in order to avoid toxic manifestations.

While effective blood schizontocidai treatment will terminate infections with *P. falciparum* and *P. malariae*, hypnozoites will in most cases continue to be present in infections with *P. vivax* and *P. ovale*. These can be eliminated by an appropriate course of a hypnozoitocidal drug.

Pharmacokinetic information is usually best for the drugs introduced after 1970. For the drugs introduced earlier the regulatory requirements usually did not stipulate such data for registration. Moreover, methods for the determination of the drugs in biological material were non-existent or too insensitive at the time when quinine and the earlier synthetic antimalarials came into wider use.

Modern procedures for the determination of drugs in biological material are mainly based on high performance liquid chromatography (HPLC) and gas chromatography (GC) with a variety of detection methods such as ultraviolet (UV) absorption fluorometry electro-chemical detection (ECD) in oxidative or reductive mode, infrared (IR) spectrography or mass spectrography[11]. Such methods also permit the study of drug partition in the blood, i.e. the assessment of drug concentrations in whole blood, erythrocytes, erythrocyte membranes, leukocytes, thrombocytes, serum and plasma. This in-

formation may be vital in the study of drug action.

Drug resistance

Drug resistance in malaria parasites has been defined as the "ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to, or higher than, those usually recommended but within the tolerability of the subject"[12]. The first reports of resistance of *P. falciparum* to quinine complying with this definition came in 1910 from Brazil[13, 14]. However, subsequently resistance to quinine proved to have little impact on therapeutic efficacy, except in parts of the Indochina Sub-Continent as from the early 1990s when massive resistance to the drug became established in the Thailand/Cambodia and the Thailand/Myanmar border areas.

Although mepacrine has been extensively used between the early 1930s and the late 1940s no case of resistance to this drug has been reported. On the other hand, it had taken not more than 2 years after the introduction of pyrimethamine that resistance to this drug has occurred both in *P. falciparum* and *P. vivax*. While this event was given little attention at the time, the advent of resistance to chloroquine of *P. falciparum* in South America and eastern Thailand in 1959 was more ominous. It initiated a race between drug development and the occurrence of resistance that is not over yet. The various stages of the race were most evident in Thailand where chloroquine was followed by sulfadoxine/pyrimethamine which was succeeded by simultaneous medication with quinine and tetracycline. This was given up in favour of mefloquine and finally this drug had to be supplemented by artemether or artesunate in order to obtain adequate therapeutic results.

In *vivo* drug response of *P. falciparum* infections to 4-aminoquinolines and 4-quinolinemethanois in non-immune hosts is commonly graded according to the classification of WHO[15]:

S Infections which clear within 5 days without recrudescence during the whole post-treatment observation period (duration depending upon the drug's half-life).

R-I Infections clearing within 5 days, negativity at least on days 6 and 7, with recrudescence during the post-treatment observation period (caveat new infections).

R-II Infections in which the asexual parasite count drops to < 25% of the initial count within 48 hours, but which fail to clear by day 7.

R-III Infections in which the parasite count fails to drop to < 25% of the initial count within 48 hours.

Drug resistance in *P. falciparum* is largely due to the selection of less sensitive and resistant parts of the parasite population under drug pressure[16]. In view of the relatively low natural frequency of mutations[17] and the lack of mutagenicity of the common antimalarials, mutation seems to play a minor role in the causation of drug resistance. In this context it should be borne in mind that the natural parasite populations consist of individuals with various levels of drug sensitivity. With regard to drugs inhibiting the parasite's metabolic pathways the distribution of these individuals according to drug sensitivity is log-concentration normal.

When a drug is used at a concentration that does not kill at least 99% of the parasite population (in non-immunes), it is likely that some parasites will survive the challenge. These will be the individuals with low sensitivity. Since drug response is a genetically determined characteristic it will be these individuals which will replace the original, and more sensitive population and build up a new one consisting mainly of parasites with reduced sensitivity or overt resistance.

Sub-critical selection pressure leading to resistance may come from mass drug administration, especially with sub-curative doses, or from inadequate treatment (inadequate doses and/or inadequate duration, often linked with compliance problems), or from the use of medicaments with a long half-life in areas with intensive malaria transmission. Here the selection pressure is exerted during the long elimination phase and directed against para-

Area	Resistance to chloroquine	Resistance to chloroquine and S/P	Resistance to chloroquine, S/P & 4-quinoline-methanols
Africa			
North	–	–	–
Tropical West	All	Coastal	Sporadic
Tropical Central	All	Focal	–
Tropical East	All	Focal	–
South	All	Focal	–
America			
South	All	All	Sporadic
Central	–	–	–
Asia			
South West	Most areas	–	–
South Central	Most areas	Focal	–
South East	All	Most areas	Myanmar, Thailand, Cambodia, Vietnam
Oceania, West	All	Most areas	–

Table 2. Drug resistance in the areas of distribution of *Plasmodium falciparum*.

Fig. 4. Geographical distribution of drug resistance in Plasmodium falciparum. Light grey: resistance to chloroquine; dark grey: resistance to chloroquine and > 10% resistance to S/P; black: resistance to chloroquine, S/P and 4-quinolinemethanols.

site populations acquired during the post-treatment period[10].

While these factors call for a rational use of antimalarial drugs it should be realized that resistance to a drug at R-I level does not essentially disqualify a medicament for further use as it may still have sufficient clinical impact to warrant its administration, especially in areas where new infections are acquired at high frequency. This applies currently to the use of chloroquine in tropical Africa, where the drug continues to be beneficial in individuals who have already acquired substantial immunity to the disease, e.g. resident adolescents and adults.

The current distribution of resistance to chloroquine, sulfadoxine/pyrimethamine, quinine and mefloquine in *P. falciparum* is given in Table 2 and shown in figure 4.

P. vivax is resistant against pyrimethamine in most areas of the species' distribution. Resistance against chloroquine has first been reported in 1989 from Papua New Guinea[18]. Subsequently it has occurred in Solomon Islands and parts of Indonesia[19, 20]. However, chloroquine-resistance of *P. vivax* has not yet become a major operational problem since quinine or mefloquine maintain high efficacy against the blood forms of *P. vivax*, and a course of primaquine would control the occurrence of relapses. In this context the dose regimen of primaquine will deserve particular attention since most reports of chloroquine-resistance originate from areas where the *P. vivax* Chesson strain prevails.

Up till now, *P. malariae* and *P. ovale* continue to respond normally to chloroquine throughout their areas of distribution so that there is no reason for using alternative medication except in cases of intolerability of chloroquine.

Compounds

4-Quinoline methanols and related drugs

Quinine

Chemistry

Quinine is 6-methoxy-α–(5-vinyl-2-quinuclidyl)-4-quinolinemethanol (figure 5), a

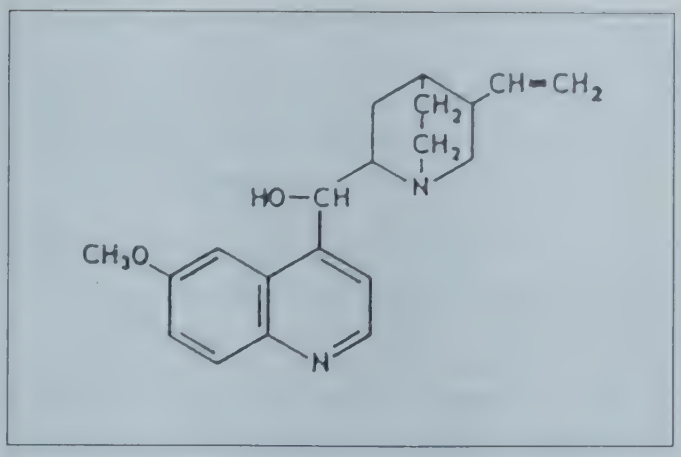

Fig. 5. Quinine.

natural main alkaloid of various species of *Cinchona* (Rubiaceae) from the bark of which it is obtained by extraction. Quinine has a molecular weight of 378.5 and is the laevorotatory stereo-isomer of quinidine. It is a white, amorphous or microcrystalline powder with very low solubility in water. It is readily soluble in methanol and ethanol. Therapeutically it is used in the form of various salts, the water-soluble of which taste intensily bitter. Aqueous solutions of quinine salts show a high degree of fluorescence.

History

The first documented use of "Peruvian bark" i.e. the bark of Cinchona trees containing quinine, for the treatment of malaria dates to the first half of the 17th century[1]. The bark rapidly found its place in many national pharmacopoeas and Cinchona plantations were started in several tropical areas outside native South America. In 1820 Pelletier and Caventon isolated the main alkaioids of the bark and described the processes for obtaining their salts[4]. Pure quinine salts soon replaced the use of the bark.

Activity in Human Malaria

Quinine exerts high blood schizontocidal activity against all specifically human-pathogenic malaria parasites (*Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*), but it is also useful in treating incidental human infections with simian plasmodia, e.g. *P. knowlesi* and *P. cynomolgi*. It is gametocytocidal in *P. vivax*, *P. ovale* and *P. malariae*, but not in *P. falciparum*. Quinine

does not possess activity against hypnozoites and pre-erythrocytic schizonts. Thus, quinine is a drug for the management of any form of clinically manifest malaria where its antiparasitic and antipyretic properties ensure a quick response. In the past quinine has also been used for suppressive treatment (prophylaxis), but this application has been discontinued when better tolerated drugs became available. Today the role of quinine is limited to the management of severe and complicated falciparum malaria requiring parenteral medication, and to the treatment of falciparum malaria resistant to chloroquine and antifols, where quinine is usually employed in association with tetracycline or doxycycline.

Pharmacology and Pharmacokinetics

The antimalarial effect of quinine is most probably due to an inhibition of the parasite's protein metabolism. In addition to the antimalarial activity, quinine exerts effects on the motor end plates of skeletal muscle, prolonging the refractory period. Quinine is a potent stimulator of the β-cells of the pancreas, thereby inducing hypoglycaemia. The often narrow difference between effective and toxic concentrations and specific pharmacokinetic characteristics of quinine necessitate the observation of very precise dose regimens.

After oral administration quinine is absorbed at > 80%, best with the hydrochloride, least with the ethylcarbonate salt[21]. Peak concentrations occur after 2-3 hours. In the steady state, with 8-hourly administration of 10 mg quinine hydrochloride per kg body weight they are usually in the range of 25-45 $\mu mol.l^{-1}$. At least 70% of the drug is bound to plasma proteins. In manifest malaria this may be increased to 90% due to enhanced binding to the acute phase α_1 acid glycoprotein. This and reduced renal function lead to a contraction of the apparent Volume of Distribution (Vd) from 1.5-1.8 $l.kg^{-1}$ in normal persons to 1.2 $l.kg^{-1}$ in acutely ill patients. The terminal plasma half-life of quinine varies between 7 and 11 hours (mean 8.7 hours) in healthy subjects, and 6-47 hours (mean 18.2 hours) in severe malaria. The area under the concentration-time curve (AUC) is therefore higher in malarious patients as compared to healthy subjects. The disease-induced alterations of the pharmacokinetics of quinine tend to disappear rapidly after the improvement of the clinical condition.

In the liver, quinine is largely biotransformed to hydroxylated metabolites. Only 20% of the drug is excreted unchanged in the urine. Faecal elimination accounts for approximately 5% of the drug. Enterohepatic circulation has been observed.

Quinine reaches the CSF in low concentration (7 ± 3% of the plasma level). It is excreted in the breast milk with a mean ratio of breast milk: plasma concentration of 0.31. A similar ratio was found in cord blood.

Resistance

The first reports of resistance to quinine in *P. falciparum*, conforming with the current definition of drug resistance at the R-I level, date from 1910 in Brazil[13, 14]. However, the phenomenon remained without operational and even therapeutic impact until the late 1980s. Although quinine is a rapidly acting drug with rather short fever clearance time (FCT) and parasite clearance time (PCT) it was – in monotherapy – always associated with a significant recrudescence rate. Emerging immunity in association with re-treatment eventually achieved radical cure in the pre-synthetics era. At present, resistance of *P. falciparum* to quinine at the R-II (very rarely R-III) level occurs in parts of the Indochina Sub-Continent, namely in Cambodia, southern Vietnam and the eastern and western border areas of Thailand.

Formulations

There are numerous formulations of quinine for oral and parenteral use. For i.v. infusion quinine hydrochloride is available in vials containing 500 mg in 1 ml, or 600 mg in 2 ml, or 1000 mg in 2 ml bidistilled water. For oral administration quinine bisulfate, quinine sulfate, quinine hydrochloride and quinine dihydrochloride are formulated as tablets, enteric-coated tablets or capsules containing 125 mg, 200 mg or 300 mg of the respective salt. Quinine ethylcarbonate, a tasteless product for paediatric use, is formulated as tablets containing 300 mg of the salt.

A solution of several *Cinchona* alkaloids in the form of their resorcinbichlorhydrates (100 mg alkaloid salt per ml), Quinimax, is also available for i.m. administration and by this route much better tolerated than the hydrochloride.

All quinine formulations contain hydroquinine in concentrations of up to 10%. This compound has antimalarial activity equal to quinine.

Dosage and Regimen

For the treatment of severe and complicated malaria quinine is used parenterally, preferably by rate-controlled intravenous infusion and under ICU conditions. In areas with a reduced sensitivity of *P. falciparum* to quinine, patients who had not been pre-treated should receive a loading dose of quinine hydrochloride of 20 mg/kg in 5% glucose solution (10 ml/kg) infused over 4 hours. In other patients the first dose will be 10 mg quinine hydrochloride per kg infused over 2 hours. Subsequent infusions with 10 mg/kg will be given at 8-hourly intervals (from start to start of the infusions) until the patient will be able to take the drug *per os* (again 10 mg/kg at 8-hourly intervals). Should fever and/or renal insufficiency persist for more than 48 hours after the start of the infusions, the quinine dose per infusion should be reduced to 2/3 or 1/2 of the regular dose, while maintaining the intervals between the infusions.

Under exceptional circumstances, suitable formulations of quinine may be given by intramuscular injection (anterior thigh), e.g. in the emergency treatment of patients with severe or complicated falciparum malaria before or during transfer to hospital. This route of administration is not considered suitable for the routine management of severe and complicated malaria.

For alternative treatment of non-severe infections with *P. falciparum* resistant to chloroquine and sulfonamide/pyrimethamine combinations, quinine hydrochloride or sulfate may be used by the oral route. The dose in patients ≥ 10 years is 10 mg/kg at 8-hourly intervals for 7 days. In patients < 10 years the same dose is given at the same interval until fever clearance. After fever clearance the individual doses are reduced to 8 mg/kg and the dose interval is shortened to 6 hours in view of the specific pharmacokinetic conditions in children. In alternative routine treatment, quinine is usually complemented by a 7-day course of doxycycline, except in pregnant women and in children < 12 years.

Side Effects and Adverse Reactions

Acute hypersensitivity reactions to quinine are relatively rare. They include skin rash, fever, asthma and thrombocytopenia and require alternative treatment with a drug other than a 4-quinolinemethanol. Blackwater fever, characterized by haemolytic anaemia, has also been considered a hypersensitivity reaction related to quinine, but the causative role of quinine is debated and the condition now recognized as an auto-immune reaction.

The most frequent side effects of quinine belong to the complex of "cinchonism" with tinnitus, nausea, headache, vertigo, auditive and visual disturbances which tend to manifest themselves with prolonged treatment. These side effects mostly resolve spontaneously after withdrawal of the medicament. Permanent hearing loss or visual deficiencies are rare but known to occur. A relatively rare adverse reaction are cardiac conduction disturbances.

Quinine is known to stimulate the β-cells of the pancreas and thereby to cause hypoglycaemia. This is particularly pronounced with parenteral treatment, the more so as severe and complicated falciparum malaria by itself is often associated with abnormally low blood glucose levels. Monitoring and correction of blood glucose concentrations is therefore part of the management of severe and complicated falciparum malaria[9].

Contraindications and Precautions

The use of quinine is contraindicated in persons hypersensitive to any of the *Cinchona* main alkaloids and in patients suffering from cardiac conduction disturbances. Quinine may cause dangerous interaction with *Digitalis* glycosides and warfarin and should not be given to patients under such treatment.

Pregnancy and early childhood are not anymore considered contraindications to treatment with quinine.

Due care is required when treating dia-

betics with quinine. This should be done under regular monitoring of the blood glucose levels also in patients receiving oral quinine for the treatment of non-severe malaria.

Quinidine

Chemistry

Quinidine is the dextrorotatory stereoisomer of quinine (figure 5) and naturally found in the bark of various species of *Cinchona* trees from which it can be extracted together with quinine, cinchonine and cinchonidine, the other main alkaloids of *Cinchona*. Quinidine has a molecular weight of 378.5 and is nearly insoluble in water. Therapeutically it is used in the form of the gluconate, bisulfate or sulfate salts. Quinidine is a white microcrystalline substance. Quinidine salt solutions show intense fluorescence.

History

As long as *Cinchona* bark was used for the treatment of malaria, quinidine was a part of the antimalarial therapy. After the pure alkaloids became available, quinine was employed for antimalarial purposes and quinidine, a class-I anti-arrhythmic agent, moved to the cardiological domain although a major clinical trial in 1866/67 had shown that all four main *Cinchona* alkaloids possess antimalarial activity[5]. Early in the 20th century studies in avian malaria suggested that the antimalarial activity of quinidine is superior to that of quinine[22], a finding reconfirmed in the 1980s with *P. falciparum* in vitro and clinically[23, 24].

Activity in Human Malaria

Although it can be assumed that quinidine acts against all human-pathogenic plasmodia, it has lately been used only in infections with *P. falciparum* where it shows rapid and intensive impact on the asexual blood forms of the parasite. It has no effect against the gametocytes of *P. falciparum*. Incidental observations in mixed infections demonstrated also blood schizontocidal and gametocytocidal activity against *P. vivax*, but no hypnozoitocidal effect in this species. The major indication for anti malarial treatment with quinidine is the emergency management of severe and complicated falciparum malaria in places where parenteral formulations of other fast-acting drugs (e.g. quinine, artesunate) are not available since there is hardly a cardiological unit that does not have a stock of quinidine ready at hand. Quinidine may also be indicated in the emergency treatment of *P. falciparum* infections with reduced sensitivity to quinine, where quinidine provides a marginal advantage.

Pharmacology and Pharmacokinetics

Quinidine is primarily used in cardiological therapy where its class-I anti-arrhythmic activity, anti-cholinergic and α-adrenoreceptor blocking effects are employed. In the context of antimalarial use, these cardiotropic activities have to be considered non-target pharmacodynamic effects the nature of which requires intensive monitoring.

Due to better solubility of quinidine gluconate or hydrochloride in water, these salts are usually employed for parenteral medication, while quinidine sulfate or bisulfate are used for oral treatment. Oral quinidine is readily absorbed (> 95%), but 20-30% undergo pre-systemic metabolism through hydroxylation. Peak concentrations in plasma are reached within 1-1.5 hours after oral administration. The cardiological therapeutic concentrations are in the range of 6-12 µmol/l. For antimalarial treatment a concentration range of 3-5 µmol/l can be considered adequate. The plasma half-life of quinidine ranges between 4 and 12 hours, with a mean of 7 hours. The Volume of Distribution (V_d) is 2-3.1 $l.kg^{-1}$ and thus less than with quinine. Approximately 70-95% of the drug is bound to plasma protein. Acute malaria increases plasma protein binding, shrinks Vd and prolongs half-life. The pharmacokinetic parameters of quinidine normalize rapidly after clinical improvement. Hepatic metabolism by hydroxylation and dihydroxylation accounts for the major elimination of quinidine of which only 15-40% are excreted unchanged in the urine.

Resistance

Quinidine shows a clear activity correla-

tion with quinine in *P. falciparum* in vitro[23], but related to the drug concentrations a 2-3 times higher effect as compared to quinine. Part of this advantage is lost due to the lesser concentration levels reached with quinidine. Thus, in areas with reduced response to quinine" also the sensitivity to quinidine is compromised, but to a lesser degree. There is still a marginal advantage of quinidine in the emergency treatment of severe and complicated falciparum malaria originating from the South-East Asian foci of multiresistance when the only other alternative drug, artesunate, is not available.

Formulations

Quinidine gluconate (Duraquin®, Quinaglute®, Quinate®) and quinidine hydrochloride are formulated as solutions in various strengths, usually 100 mg or 200 mg per ml, for i.m. injection or rate-controlled i.v. infusion. Quinidine sulfate (CinQuin®, Kinidine®, Quinicardine®, Quinidex®, Quinidoxin®, Quinora®, Systodin®, and slow-release formulation Optochinidin®) and quinidine bisulfate (Biquin®, Kiditard®, Kinidin Durules®, Quinidurile®) are formulated as tablets, usually containing 100 mg, 200 mg or 250 mg of the salts.

Dosage and Regimen

Given the current possibilities for the treatment of uncomplicated malaria, even in areas with multidrug-resistance of *P. falciparum*, quinidine is only indicated for the emergency treatment of severe and complicated falciparum malaria in areas where quinine or artesunate are not available. In view of the potential cardiovascular side-effects of quinidine the initial treatment should be carried out at an intensive care facility, with cardiovascular monitoring. In view of the different base content of quinidine gluconate and quinidine hydrochloride, the quinidine doses are calculated on the base content. A loading dose of 15 mg quinidine base/kg body weight by rate-controlled i.v. infusion over 4 hours (in 10 ml/kg physiological saline or 5% glucose solution) is indicated in patients who contracted the infection in an area with reduced quinine sensitivity and who had not taken any quinine or quinidine during the preceding 24 hours, or mefloquine during the preceding 4 weeks. In these cases, and in patients coming from areas with normal quinine sensitivity, the initial dose is 7.5 mg quinidine base/kg, infused within 2 hours.

Subsequent infusions are given at 8-hourly intervals (start to start of infusion), with 7.5 mg quinidine base/kg and a running time of 4 hours. Quinidine doses should be reduced by one-third to one-half on the third day of treatment in the absence of an overall improvement of the clinical condition or if renal failure has developed. Parenteral medication should be replaced by oral therapy as soon as possible, completing the seven-day course with quinine or quinidine. If quinidine is used for the oral medication, 6-hourly doses of 8 mg quinidine sulphate or quinidine bisulphate (calculated as salt) are required to compensate for the relatively short half-life of the drug. In order to reduce the risk of recrudescence, this should be followed by a seven-day course of doxycycline, adult dose 100 mg once daily (not in children < 12 years of age, and pregnant women).

Side Effects and Adverse Reactions

Acute hypersensitivity to quinidine is rare and manifests usually as exfoliative dermatitis or granulomatous hepatitis. In patients with heart conditions quinidine should be avoided if other effective medication is available. Otherwise it should be given with utmost caution and under continuous monitoring of ECG and blood pressure. Specific risks in patients with cardiovascular conditions are heart block, cardiac arrhythmias and heart failure.

Nausea, vomiting and diarrhoea are the most frequent side effects of quinidine. They are of minor importance while parenterai medication is given and usually occur after changing to oral therapy. The use of sustained release formulations can be beneficial in these cases. Cinchonism with impaired hearing, headache, dizziness, blurred vision and vomiting is a frequent event in the later part of a quinidine regimen. It usually resolves spontaneously after the therapy. Milder hypersensitivity may manifest in the form of urticaria and an exacerbation of psoriasis and other pre-existing skin conditions. Quinidine

stimulates the β-cells of the pancreas and may lead to hypoglycaemia or enhance the hypoglycaemia often seen in severe or complicated falciparum malaria. Monitoring of the glucose levels and corrective action are therefore required while the patient receives quinidine. The drug may aggravate myasthenia gravis due to its depressant action on neuromuscular transmission.

Contraindications

The use of quinidine for antimalarial treatment is contra-indicated in patients with cardiovascular diseases and in infants. There is little experience with quinidine in children. If at all, quinidine should in this group be used with great caution. Until one month before term, pregnancy is no contraindication to treatment with quinidine.

Mefloquine

Chemistry

Mefloquine is α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol (figure 6) and obtained by chemical synthesis. It has a molecular weight of 379.3 for the free base and 414.8 for the hydrochloride which is used for the pharmaceutical formulations, Mefloquine has dextrorotatory and laevorotatory enantiomers, but it is formulated as racemate. Mefloquine hydrochloride is a white, amorphous and odourless powder. It has a slightly bitter, brackish taste and is readily soluble in ethanol and methanol. Solubility in water is 1 in 250.

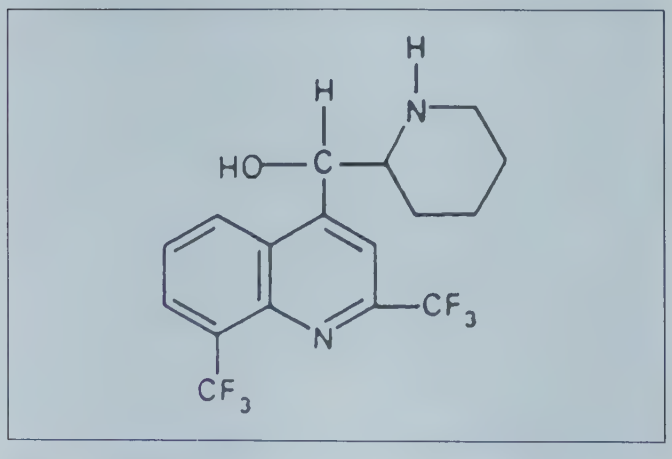

Fig. 6. Mefloquine.

History

The antimalarial activity of synthetic quinolinemethanols was known since World War II when such compounds were tested in the course of a drug screening programme in the USA[25]. However, further development was discontinued due to toxic reactions observed with the early trial drugs. Later new members of this chemical class were explored by the Walter Reed Army Institute of Research (WRAIR) which, in the early 1970s, identified compound WR 142 490 (mefloquine) as the most suitable candidate drug. As from 1976 mefloquine has been developed by WHO in collaboration with WRAIR and Hoffmann-La Roche. In 1984 the drug was registered under the name of Lariam®.

Activity in Human Malaria

Mefloquine has rapid and high blood schizontocidal activity in all primate, rodent and avian plasmodia so far tested. The drug has been primarily developed as an alternative medicament for the treatment of chloroquine-resistant *P. falciparum*, but from experience with mixed infections it is known to be also effective against *P. vivax* and *P. malariae*, where it has also gametocytocidal activity which it lacks for *P. falciparum*. It has no effect against pre-erythrocytic schizonts and hypnozoites. The blood schizontocidal activity of mefloquine is most marked at the trophozoite stage and independent of that of chloroquine and sulfonamide/pyrimethamine combinations. However, there is evidence of cross-sensitivity or cross-resistance, respectively, with quinine in *P. falciparum*[26]. An inverse relationship between the sensitivity to chloroquine and mefloquine in *P. falciparum* has been reported[27].

Pharmacology and Pharmacokinetics

The antimalarial effect of mefloquine is believed to be due to an inhibition of acid proteases and peptidases in the parasite's food vacuoles. Mefloquine has a high affinity for erythrocyte membranes. It is also selectively taken up by intraerythrocytic malaria parasites. As a non-target effect, mefloquine inhibits acetylcholinesterase and buturylcholinesterase. This may explain the relatively frequent gastro-intesti-

nal side effects such as vomiting and diarrhoea, and adverse reactions involving the CNS. Mefloquine is not mutagenic. Little is known about differential activity and tolerability of the stereo-enantiomers.

Earlier observations with pure mefloquine hydrochloride in the form of an aqueous suspension or capsules indicated very rapid absorption, commencing in the pylorus. This was associated with very sudden side effects, especially vomiting and diarrhoea. Therefore the drug has been formulated for staggered absorption. After the oral administration of Lariam® tablets, about 75-80% of the drug is absorbed, with tmax values of 2-12 hours. There is little pre-systemic metabolism. The plasma $t_{1/2}$ varies between 15 and 33 days, with a mean of 21.4 days, the longest so far known from any antimalarial. Mefloquine is rapidly distributed throughout the body, with an apparent V_d of 19.2 ± 7.0 $l.kg^{-1}$ in healthy volunteers, and high affinity to lipid tissues. In the blood plasma a large part of mefloquine is protein-bound (> 98%). This may also account for the inability of mefloquine of crossing the blood-brain barrier. Mefloquine is mainly metabolized in the liver and only 9% of the parent drug are eliminated, unchanged, in the urine. The bulk is excreted with the faeces in the form of the main metabolite, 2,8-bis(trifluoromethyl)-4-quinoline carboxylic acid (briefly called carboxy-mefloquine), which has no antimalarial activity, a toxicity similar to that of the parent compound, and a terminal half-life exceeding that of mefloquine. Carboxy-mefloquine becomes detectable 4 hours after the intake of mefloquine. The plasma concentrations continue to rise and soon exceed those of the parent compound. A marked contraction of the first compartment with reduction of V_d and an increase of C_{max} is seen in malarious patients. Elimination half-life of mefloquine is reduced in malaria patients as compared to healthy subjects. Values of $t_{1/2}$ in malarious children are inferior to those observed in healthy adults[28].

Peak plasma concentrations of mefloquine in healthy adult Thai males of approximately 50 kg body weight following a therapeutic single dose of 250 mg (base) were in the range of 1.7 and 6.6 $\mu mol.l^{-1}$. In Thai malarious patients treated with the same dose they were between 2.6 and 8.0 $\mu mol.l^{-1}$ [29].

Due to the long elimination half-life of mefloquine, steady state with the weekly administration of the prophylactic dose of 250 mg mefloquine (base) is mostly reached within 8-10 weeks. In steady state the plasma concentration of mefloquine is usually above 2 $\mu mol.l^{-1}$, with a mean mefloquine: carboxy-mefloquine ratio of 1:5.6.

Mefloquine is excreted in the breast milk, but the excreted quantities of the drug are too small for exerting any therapeutic or prophylactic activity in the infant.

Resistance

Sporadic failure of treatment or prophylaxis with mefloquine has been reported from various parts of the world, especially from West Africa, but supportive pharmacokinetic information is lacking in most cases, However, *in vitro* data from West Africa suggest that some isolates of *P.falciparum* show low primary sensitivity, a phenomenon that may be related to the widespread practice of inadequate treatment with quinine.

Since the early 1990s resistance of *P. falciparum* to mefloquine has become an operational problem in parts of the Indochina Sub-Continent, namely in Cambodia, the southern parts of Vietnam (although the drug has not been used there to any significant extent), and in the Thailand/Myanmar and the Thailand/Cambodia border areas. The relatively early occurrence of resistance to mefloquine may be due, in part, to an a *priori* reduced sensitivity as a result of poor response to quinine, but the major factor obviously was post-treatment selection pressure in areas with intensive malaria transmission in Cambodia and Myanmar[30].

Formulations

Mefloquine is formulated in cross-scored tablets containing 273 mg of the hydrochloride, corresponding to 250 mg base. The two commercial formulations (Lariam®, Hoffmann-La Roche and Mephaquin®, Mepha) have different bioavailability[31], with

Mephaquin® showing lesser AUC values and higher tolerability. Mefloquine is also formulated in a fixed combination with sulfadoxine and pyrimethamine (Fansimef®, Hoffmann-La Roche) in tablets containing 250 mg mefloquine, 500 mg sulfadoxine and 25 mg pyrimethamine. There is no formulation for parenteral use.

Dosage and Regimen

For therapeutic use mefloquine is given at an adult dose of 12.5-15.0 mg/kg body weight up to a total dose of 1500 mg. Up to 750 mg (3 tablets) may be given as a single dose. Higher doses should be split (e.g. for the maximum dose 750 mg, 500 mg, 250 mg at intervals of 8 hours). The dose per weight is higher in children (5-9 years: 20 mg/kg). For children < 5 years the dose should be calculated according to body surface, using Dubois' formula

$$\text{Body surface } (m^2) = \text{length } (cm)^{0.725} \times \text{weight } (kg)^{0.425} \times 0.007184$$

and a dose of 600 mg/m^2 body surface.

For suppressive cure (prophylaxis) mefloquine is given as a weekly adult dose of 250 mg (1 tablet), starting 1 week before exposure and continuing for 4 weeks after exposure to malaria transmission has ended. Although medium term use, up to three years, seems to be safe[32] it is still debated whether a loading dose is required in order to render early protection more reliable and whether a dose reduction is indicated after 5-8 weeks as to avoid steady state concentrations at toxic level.

The weekly prophylactic doses for children and juveniles are as follows:

15-24 kg body weight	1/4 tablet (62.5 mg)
25-34 kg body weight	1/2 tablet (125 mg)
35-44 kg body weight	3/4 tablet (197.5 mg)
≥ 45 kg body weight	1 tablet (250 mg)

Side effects and Adverse Reactions

Acute hypersensitivity to mefloquine is rare and manifests as urticaria, rash or pruritus. By far the most frequent side effects associated with therapeutic doses are dizziness, vertigo, nausea, vomiting, diarrhoea and headache, but in the individual patient it may be difficult to differentiate them from the symptoms of acute malaria. Such side effects occur in approximately 40% of the patients. They are dose-dependent. In addition, sinus bradycardia is frequently observed, especially after the resolution of the clinical symptoms of malaria. Although much less frequent, neuro-psychiatric adverse reactions do occur. They are dose-dependent and range from acute brain syndrome to depression, paranoid and schizoid reactions. They may occur early after dosing, but often they manifest only in the second or third week after treatment and usually resolve without sequelae.

Under prophylaxis side effects are similar In type, however of lesser frequency except for nightmares and sinus bradycardia which are rather common. Side effects usually manifest within the first four weeks after starting prophylaxis.

Mefloquine may interfere with the efficacy of oral typhoid vaccines. Immunization with such vaccines should therefore be completed before starting prophylaxis with mefloquine.

Contraindications and Precautions

Mefloquine is contraindicated in patients who have been treated with quinine within the past 24 hours or with halofantrine within the past 7 days. It is also contraindicated in persons with a history of epilepsy, seizures or psychoses.

Persons with occupations requiring a high degree of spacial perception and coordination (e.g. pilots) should not receive prophylaxis with mefloquine. After treatment with mefloquine they should not resume their occupational activities for 4 weeks.

Pregnancy in the 2nd and 3rd trimester is no contraindication to the therapeutic or prophylactic use of mefloquine. Although treatment with mefloquine during the first trimester of pregnancy can not anymore be considered a justification for therapeutic abortion, it should be avoided and a suitable alternative drug used (e.g. quinine). Similarly, prophylaxis with mefloquine in women of child-bearing age should be carried out under contraception (to be continued until two months after the last dose). Earlier suspicions regarding an interaction between mefloquine and cardiovascular drugs, especially β-blockers, were not sustained.

Halofantrine

Chemistry

Halofantrine is 1,3-dichloro-α-(2-(dibutylamino)ethyl)-6-(trifluoromethyl)-9-phenanthrenemethanol (figure 7) and obtained by chemical synthesis. It has a molecular weight of 500.4 for the free base and 536.9 for the hydrochloride which is used therapeutically. Halofantrine has dextrorotatory and laevorotatory enantiomers, but the commercial formulation contains the racemate. Halofantrine hydrochloride is a microcrystalline or amorphous, yellowish powder and practically insoluble in water. It can be dissolved in absolute methanol and ethanol.

History

Halofantrine has been developed at the Walter Reed Army Institute of Research (WRAIR), Washington, D.C., in the 1970s. Later, industry joined in the development (SmithKline Beecham) and registered the drug in 1988 under the name of Halfan®.

Activity in Human Malaria

Halofantrine exhibits marked blood schizontocidal activity in the *P. berghei* mouse model which was also used for the antimalarial screen from which the drug emanated as a candidate compound. The activity was confirmed in primate malarias including human infections with *P. falciparum* and *P. vivax*. It has no effect against pre-erythrocytic forms of plasmodia and against gametocytes of *P. falciparum*. The blood schizontocidal effect of halofantrine is independent of that of chloroquine and antifols. However, it has marked cross-sensitivity/cross-resistance with mefloquine[33]. Halofantrine has been developed as an alternative drug for the treatment of *P. falciparum* infections resistant to 4-aminoquinolines and antifols.

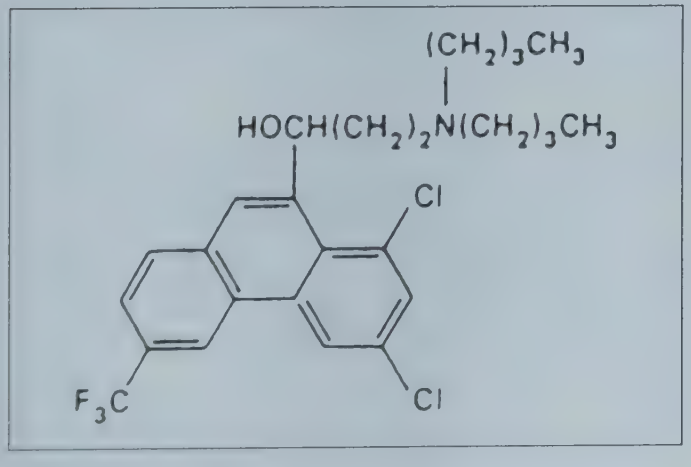

Fig. 7. Halofantrine.

Pharmacology and Pharmacokinetics

The antimalarial activity of halofantrine is directed against the trophozoite stage and apparently due to the inhibition of acid proteases and peptidases. Little is known about partition between the various blood components and about the specific activity and toxicity of the stereo-enantiomers.

In the absence of an intravenous formulation of halofantrine it was impossible to measure the relative bioavailability of the drug. From available data it appears that absorption after oral administration is highly variable and subject to a window effect suggesting that quantities in excess of a critical limit may not be absorbed. C_{max} is usually reached within 6-10 hours. There is little pre-systemic metabolism. The plasma half-life after three doses of 500 mg given at 6-hourly intervals in Thai patients suffering from falciparum malaria was 113 ± 31 hours[34].

In the same patients the mean C_{max} was 1192 ± 410 mg/ml corresponding to 2.38 ± 0.82 $\mu mol.l^{-1}$, and the AUC was 60.6 + 23.9 $\mu g.h.ml^{-1}$. These data suggest considerable inter-individual variability which was also evident from the treatment results inasmuch as the cure rate was better in patients with higher C_{max} and AUC values as compared to those with low C_{max} and AUC ratings. There is evidence that the absorption of halofantrine is markedly enhanced if the drug is taken with fatty food[35].

Halofantrine is metabolized in the liver, with monodesbutyl-halofantrine as the main metabolite which reaches C_{max} approximately 12 hours after dosing. The bulk of halofantrine and its main metabolite is eliminated with the faeces. Renal excretion accounts for not more than 1% of the drug intake.

Resistance

True resistance of *P. falciparum* to halo-

fantrine has so far only been observed in areas of the Indochina Sub-Continent where the species is also resistant to mefloquine. Although poor *in vitro* response of *P. falciparum* occurs sporadically also in other parts of the world, therapeutic failure in those regions is predominantly due to pharmacokinetic factors, especially poor absorption.

Formulations

Halofantrine (Halfan®) is formulated as tablets containing 250 mg halofantrine hydrochloride (corresponding to 233 mg halofantrine base) and as a suspension containing 20 mg halofantrine hydrochloride per ml.

Dosage and Regimen

Indications for the use of halofantrine are therapeutic only (not for chemoprophylaxis). Persons with a body weight ≥ 40 kg receive 3 doses of 500 mg (2 tablets) each at intervals of 6 hours. Children are given 3 doses of 12 mg/kg body weight each at 6-hourly intervals. In non-immunes the same course is repeated one week later (NB: not earlier!). Whenever possible the drug is to be given on an empty stomach, but in no case with fatty food.

Side Effects and Adverse Reactions

Halofantrine is generally well tolerated, but gastrointestinal symptoms such as nausea, vomiting, diarrhoea and abdominal pain, and transient increase of serum transaminases, may occur in 15-25% of the patients. Headache and rash are rarely caused by the medication and mostly due to the underlying disease. With the use of the suspension hypersensitivity reactions to sodium benzoate may be observed. By far the most important adverse effects are orthostatic hypotension and a prolongation of the QTc interval which is clearly related to the blood concentration of halofantrine and strongest at C_{max}[36].

Such prolongations of the QTc interval may be present in up to 50% of the patients, but they will usually not lead to dangerous, eventually fatal, manifestations except in patients with a pre-existing prolongation of the QTc interval or in patients who had received two or three treatment courses in short succession[36].

Contraindications and Precautions

Halofantrine is strongly contraindicated in persons who have taken mefloquine, whether for treatment or prophylaxis, within the past 8 weeks, or quinine or quinidine within the past 48 hours. Similarly, halofantrine is contraindicated in patients with prolonged QTc interval and/or history of cardiovascular conditions. Halofantrine should not be given in pregnancy.

Whenever possible, treatment with halofantrine should be given under close medical supervision, preferably in hospital.

4-Aminoquinolines

Chloroquine

Chemistry

Chloroquine is 7-chloro-4-(4'-diethylamino-1'-methylbutylamino) quinoline (figure 8) and obtained by chemical synthesis which yields the racemate of the dextrorotatory and laevorotatory enantiomers. Chloroquine (base) has a molecular weight of 319.9 and is poorly soluble in water. Therapeutically it is used in the form of the phosphate, sulfate or hydrochloride salts which are readily soluble in water. Chloroquine and its salts are white odourless powders with an intensily bitter taste.

History

The 4-aminoquinolines began to be explored shortly before the second World War. Initially the exploration of chloroquine was erroneously discontinued in Ger-

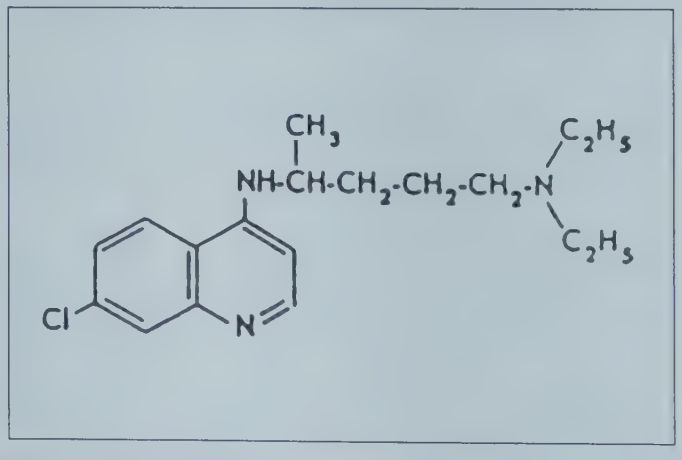

Fig. 8. Chloroquine.

many, but further work in the U.K. and the USA proved that it was the most effective and best tolerated synthetic antimalarial compound so far tested. The drug was introduced for wider use in the late 1940s.

Activity in Human Malaria

Chloroquine is a blood schizontocidal drug that acts against all species of human-pathogenic plasmodia, including those generally restricted to non-human primates and only occasionally infecting man (e.g. *P. knowlesi* and *P. cynomolgi*) . Similar to the 4-quinolinemethanols it exerts its main activity in the trophozoite stage. Chloroquine destroys also the gametocytes of *P. vivax*, *P. malariae* and *P. ovale*, whereas it lacks gametocytocidal activity in *P. falciparum*. Chloroquine has no activity against hypnozoites and pre-erythrocytic schizonts. Therapeutic and prophylactic activity of chloroquine against *P. falciparum* in large parts of the tropics and against *P. vivax* in parts of Melanesia is now compromised by resistance.

Pharmacology and Pharmacokinetics

The most important pharmacodynamic effect of chloroquine is its antimalarial activity, followed by anti-inflammatory properties. The drug has high affinity to melanin. At concentrations well above the therapeutic level it reduces excitability and conductivity of the heart muscle, exerting thereby a depressant effect manifesting with bradycardia or heart arrest. Various hypotheses have been developed to explain the mechanism of antimalarial activity of chloroquine. While it seems clear that the drug inhibits acid proteases and peptidases in the food vacuoles and that its activity depends on getting to and staying at these sites, other hypotheses are still debated.

After oral administration chloroquine is absorbed rapidly (t_{max} = 1-3 hours) and to nearly 100% also in the presence of diarrhoea. The drug is rapidly distributed throughout the body, with the relatively high mean apparent V_d of 200 $l.kg^{-1}$. This explains also the relatively low plasma concentrations observed with therapeutic doses. However, there are marked differences between the chloroquine concentrations in the various blood components. The chloroquine concentration in uninfected erythrocytes is approximately 4-5 times of that in plasma. Erythrocytes infected with chloroquine-sensitive *P. falciparum* show about 500 times the plasma concentration. Similarly, chloroquine concentrations in leukocytes and thrombocytes are approximately 500-1000 times higher than those in the plasma. Upon coagulation the thrombocytes loose chloroquine. This explains why chloroquine concentrations in serum are higher than those measured in the plasma. Approximately 50-70% of the chloroquine in blood plasma is protein-bound.

Due to the abundant peripheral distribution of chloroquine and the time required for reaching equilibrium between the central and peripheral compartments one differentiates between the half-life in the initial elimination phase (2-6 days) and terminal elimination half-life (30-60 days). In the context of therapeutic efficacy of chloroquine only the half-life of the initial elimination phase is of importance. Part of the chloroquine is metabolized in the liver to monodesethyl-chloroquine which has an antimalarial activity similar to that of chloroquine. A small part is further metabolized to bidesethyl-chloroquine and a primary amine, both of which lack antimalarial activity. Elimination of chloroquine and its metabolites is mainly through the urine, a much smaller part is excreted in the faeces.

Resistance

Resistance of *P. falciparum* to chloroquine has been first observed in 1959 almost simultaneously in South America and in the border area of Thailand and Cambodia[7, 8], both sites near areas where chloroquinated salt was used for malaria control[37]. Subsequently large areas of eastern Asia, western Oceania and South America became involved, and from 1977 also tropical Africa[38, 39]. By 1996 the area of distribution of chloroquine-resistant *P. falciparum* has become identical with that of the species except in America north of the Panama Canal and a few areas in Southwest Asia and North Africa. Recently resistance of *P. vivax* to chloroquine has been reported from Melanesia[18, 19, 20, 40], but in the

major areas of distribution the species continues to show satisfactory response to chloroquine. As a result of resistance chloroquine has been replaced as a first line drug for the treatment of falciparum malaria in Asia and South America. In tropical Africa it continues to provide adequate clinical relief in semi-immunes, but for the treatment of infants and young children it has been replaced in many areas.

Formulations

Chloroquine is mainly formulated for oral use, but there are also formulations for parenteral administration. Tablets of chloroquine diphosphate (Aralen®, Avloclor®, Delagil®, Resochin®) contain 150 mg, 100 mg or 50 mg base. Tablets of chloroquine sulfate (Nivaquine®, Nivaquine B®) contain 100 mg or 300 mg base. There is also a syrup of chloroquine sulphate for paediatric use (Nivaquine® syrup) containing 10 mg chloroquine base per ml.

The injectable formulations contain chloroquine sulfate (Nivaquine® for injection) or chloroquine hydrochloride (Aralen® for injection). The ampoules (5 ml) contain 40 mg chloroquine base/ml.

Dosage and Regimen

For therapeutic use in non-severe falciparum malaria and in infections with *P. vivax* and *P. ovale* chloroquine is given by the oral route at a total dose of 25 mg (base) per kg body weight over 3 days. The following standard regimen is most widely used:

Day 1	10 mg(base)/kg
8 hours later	5 mg(base)/kg
Day 2	5 mg(base)/kg
Day 3	5 mg(base)/kg

Oligosymptomatic infections may be treated with 10 mg(base)/kg on the first and second day, followed by 5 mg(base)/kg on the third day. In quartan malaria (*P. malariae*) the total dose of chloroquine is 35 mg(base)/kg over 5 days: first day 10 mg(base)/kg, second day 10 mg(base)/kg, third to fifth day each 5 mg (base)/kg .

In severe and complicated falciparum malaria chloroquine may be administered parenterally if no other, more suitable medicament is available and where the parasites have not yet reached a high level of resistance to chloroquine.

In such cases administration by rate-controlled continuous intravenous infusion is preferable. The first dose, 10 mg(base)/kg in isotonic infusion fluid (10 ml/kg), should be given over 8 hours, followed by 15 mg(base)/kg infused over the next 24 hours. Precise adjustment of infusion speed is indispensable in order to avoid dangerous adverse reactions. If chloroquine is administered by the intramuscular route due care is required not to exceed a dose of 3.5 mg(base)/kg. This dose may be given at 6-hourly intervals until a total dose of 25 mg(base)/kg has been reached.

NB: radical treatment of vivax- and ovale-malaria requires additional treatment with primaquine.

For chemoprophylaxis chloroquine is used at a weekly dose of 5 mg(base)/kg, usually starting 1 week before exposure and continuing for 4-6 weeks after exposure. A loading dose of 5 mg(base)/kg on the second day of prophylaxis accelerates the establishment of protective steady state concentrations. "Enhanced" prophylaxis using an adult dose of 100 mg(base) per day on 6 days of the week is also practised, but only suitable for short-term use.

Chloroquine is also used for the treatment of diseases other than malaria, employing different dose regimens. These are not mentioned in this context.

Side Effects and Adverse Reactions

The most frequent and spontaneously resolving adverse effects observed with normally dosed oral treatment are nausea, vomiting, headache, dizziness, abdominal pain, diarrhoea and rash. Itching is a frequent complaint among African patients. Visual disturbances such as difficulties of accomodation and blurring of vision may occur under normal treatment and should not be confused with retinopathy, a non-reversible serious adverse effect associated with the long-term use of the drug. The risk of retinopathy starts at a cumulative intake of 100 g chloroquine (base) and is also dependent on the time over which the cumulative dose was taken. Retinopathy usually leads first to a narrowing of peripheral vision, and finally to blindness if

chloroquine is not withdrawn early enough. Retinopathy is believed to be caused by the high affinity of chloroquine to melanin in the retina. Other adverse effects associated with the long-term use of chloroquine are a pigmentation of the hard palate, reversible bleaching of hair, and skin eruptions. Life-threatening and fatal adverse reactions may occur with faulty parenteral administration of chloroquine, overdoses and poisoning, manifesting with severe postural hypotension, heart block and cardiopulmonary arrest. Also the CNS may be affected (convulsions, psychosis). Auditive and visual defects have been observed in children born to women who had taken high doses of chloroquine during pregnancy, usually for the treatment of rheumatoid arthritis.

Interaction between chloroquine and verapamil or desipramine in *P. falciparum* in *vitro* was shown to reverse chloroquine resistance[41, 42], but clinical observations with verapamil in patients suffering from chloroquine-resistant falciparum malaria yielded no evidence of the same effect *in vivo*[43].

Contraindications and Precautions

There is an absolute contraindication for the administration of chloroquine by intravenous bolus injection. The same applies to the intramuscular injection of doses exceeding 3.5 mg (base)/kg. Known hypersensitivity to chloroquine is also a contraindication to its use.

Treatment with chloroquine in patients with a history of epilepsy, seizures, psychosis, or of severe cutaneous reactions to other drugs should be conducted cautiously and preferably in hospital.

There are no contraindications to treatment or prophylaxis with chloroquine in normal pregnancy and in infancy.

Amodiaquine

Chemistry

Amodiaquine is 7-chloro-4-(3'-diethylaminomethyl-4' -hydroxyanilino)-quinoline (figure 9), a Mannich base and analogue of chloroquine. It is obtained by chemical synthesis and has a molecular weight of 355.9 for the free base, and 464.8 for the

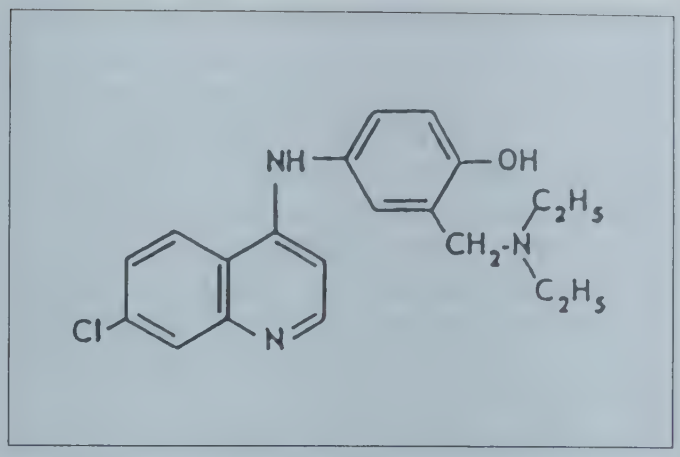

Fig. 9. Amodiaquine.

dihydrochloride dihydrate which is used for the pharmaceutical formulations. The salt is a yellow crystalline powder which is soluble in water (1:223 and in ethanol (1:70).

History

Amodiaquine is one of the 4-aminoquinolines developed during World War II and coming into wider use in the late 1940s, but due to its higher price it never became as popular as chloroquine. After the onset of resistance of *P. falciparum* to chloroquine, the drug was increasingly employed for prophylaxis. However, the incidence of serious adverse reactions was such that the prophylactic use has been discontinued as from 1986. Although it has been recommended to extend this also to the therapeutic use of amodiaquine[44] the drug continues to be employed in endemic areas, especially in tropical Africa.

Activity in Human Malaria

The type of activity of amodiaquine in human malaria is almost identical with that of chloroquine. However, the efficacy is slightly higher, so that amodiaquine may be still effective against *P.falciparum* with low-grade resistance to chloroquine.

Pharmacology and Pharmacokinetics

Apart from the specific blood schizontocidal activity in plasmodia little has been reported about other pharmacodynamic effects of amodiaquine. The antimalarial mechanism of action seems to be quite similar to that of chloroquine.

After oral administration amodiaquine is rapidly and almost completely absorbed, reaching C_{max} after 30 minutes[45]. The drug is subject to extensive presystemic metabolism yielding monodesethyl-amodiaquine which is as active as the parent drug. There is controversy about the speed with which amodiaquine disappears from the blood[46]. This may be related to the HPLC assay methods used and/or the origin of the subjects. In the first study, carried out in Caucasians, amodiaquine was detectable for 8 hours after oral administration. In another study, in Africans, no amodiaquine was detectable at all and only monodesethyl-amodiaquine was found, with C_{max} (approximately 240 $\mu g.l^{-1}$ in plasma) between 90 and 120 minutes after drug intake[47]. The concentration of monodesethyl-amodiaquine in whole blood is 4-6 times higher than in plasma, pointing to marked uptake by the cellular blood components. Such partition was not seen with amodiaquine.

The pharmacokinetic findings suggest that amodiaquine is a prodrug of the equally effective monodesethyl-amodiaquine. There is, unfortunately, no information on differential toxicology of these two compounds. The peripheral distribution of monodesethyl-amodiaquine is high and similar to chloroquine, but there is a considerable difference between the initial elimination phase half-life (23-50 hours) and the terminal elimination half-life (9-18 days). Pre-systemic metabolism may already take place in the intestinal wall, but most of it is likely to take place in the liver which is also the site of post-systemic metabolism. The metabolic transformation to monodesethyl-amodiaquine is rapid. There is further, partial, transformation to 2-hydroxydesethyl-amodiaquine. Elimination is mainly renal.

Resistance

Resistance of *P. falciparum* to amodiaquine has been reported soon after the advent of resistance to chloroquine. There is marked cross-sensitivity/cross-resistance between these compounds, but the relative activity of amodiaquine or monodesthyl-amodiaquine is higher (also on the basis of the blood concentration). This explains the phenomenon that amodiaquine retains therapeutic efficacy longer than chloroquine. In areas with high levels of resistance to chloroquine and non-immune population there will be no therapeutic benefit from amodiaquine while the situation is obviously different in areas where the drug effect is complemented by partial immunity, e.g. tropical Africa.

Formulations

Amodiaquine dihydrochloride is formulated in tablets containing 150 mg or 200 mg of amodiaquine base (Camoquine®, Flavoquine®, Miaquine® and generics). The production of injectable formulations of amodiaquine has been discontinued.

Dosage and Regimen

The only indication for the use of amodiaquine is therapeutic in situations where the drug offers an advantage over treatment with chloroquine. The oral dose regimen is identical with that of chloroquine.

Side Effects and Adverse Reactions

The most frequent side effects associated with the therapeutic use of amodiaquine are nausea, anorexia, vomiting, abdominal cramps and diarrhoea. Hypersensitvity reactions to amodiaquine are rare. Such hypersensitivity usually extends to other 4-aminoquinolines.

Liver damage, leucopenia and agranulocytosis, in some cases fatal, have been observed under malaria prophylaxis with amodiaquine and under prolonged treatment for rheumatoid arthtitis. Pre-existing hepatic insufficiency seems to enhance the risk of agranulocytosis. In the absence of toxicological studies with amodiaquine and monodesethyl-amodiaquine it is not known whether these serious adverse reactions may be due to reduced metabolic capability/capacity.

Contraindications and Precautions

Prophylactic use of amodiaquine is contraindicated. So is the repetition of therapeutic courses of amodiaquine at short interval. Patients with hepatic dysfunction or a history of leucopenia following the use of other medicaments should not be given amodiaquine. Since little is known about interactions between amodiaquine

and other drugs, amodiaquine should not be given in association with or parallel to other medicaments, the more so as drug interaction could not be excluded as cause or aggravating factor in the majority of patients who had suffered from serious adverse reactions to amodiaquine.

8-Aminoquinolines

Primaquine

Chemistry

Primaquine is 6-methoxy-8-(4'-amino-1'-methylbutylamino) quinoline (figure 10). It is obtained by chemical synthesis which yields the racemate of the dextrorotatory and laevorotatory enantiomers. Primaquine base has a molecular weight of 259.4. Therapeutically it is used in the form of the diphosphate (molecular weight 455.3) which is soluble in water 1:16. Primaquine diphosphate is an orange-red crystalline substance.

History

The first 8-aminoquinoline with antimalarial activity, pamaquine, has come into use in the late 1920s as a potential replacement of quinine in the treatment of clinical malaria. In this application it did not fulfill the expectations due to relatively low blood schizontocidal activity in human malaria and substantial toxicity. Later it was found that pamaquine has radical curative properties in vivax- and ovale-malaria where it has hypnozoitocidal activity. Primaquine, a better tolerated analogue of pamaquine, was developed during World War II and introduced in the late 1940s.

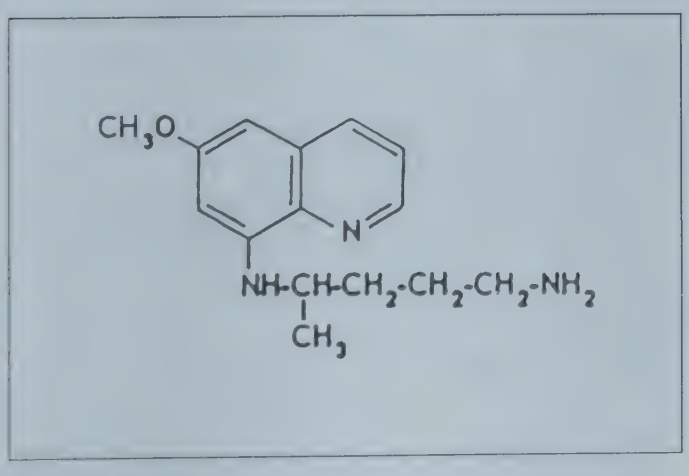

Fig. 10. Primaquine.

Activity in Human Malaria

Primaquine has tissue schizontocidal and moderate blood schizontocidal activity in all species of human-pathogenic plasmodia but its relatively high toxicity precludes its use for such purposes. Primaquine has marked activity against the hypnozoites of *P. vivax*, *P. ovale* and *P. cynomolgi*, and gametocytocidal activity in all human plasmodia as well as sporontocidal activity in *P. falciparum*. The short half-life and the scanty elimination of primaquine and the observation that the total dose level rather than the dose regimen determines hypnozoitocidal efficacy[48] suggest that one or several metabolites of primaquine are responsible for the biological activity of the drug. The two stereo-enantiomers of primaquine have similar biological activity.

Pharmacology and Pharmacokinetics

Primaquine and some of its metabolites inhibit mitochondrial respiration of the parasite and disrupt pyrimidine biosynthesis. In addition to these antiparasitic properties. primaquine is known to stimulate the oxidative pathway of glucose metabolism in the normal erythrocyte[49]. Protective mechanisms against this oxidative stress are inadequate in G6PD deficiency and it may come to haemolysis in individuals suffering from this trait. Unusually high doses of primaquine may cause methaemoglobinaemia and cyanosis, especially in NADH methaemoglobin reductase-deficient persons.

After oral administration primaquine is quickly and almost completely absorbed, reaching C_{max} within 2 hours. There is extensive pre-systemic metabolism. The plasma half-life is 5.3-8.1 hours.

The drug is widely distributed, the renal clearance is low. This explains the minimal renal elimination of primaquine as parent drug (< 1% within the first 24 hours). The large majority is converted to metabolites which have a much longer half-life as compared to primaquine, explaining the protracted biological activity mentioned above. Metabolism of primaquine takes mainly place in the liver. It transforms more than 99% of the drug into a variety of

metabolites, especially 5-hydroxy, 6-methoxy and 6-dihydro derivatives which are largely eliminated in the urine. Both the therapeutic effect and the haemolytic activities in G6PD deficient individuals seem to be due to metabolites rather than the parent substance.

Resistance

Although no resistance of *P. vivax* hypnozoites to primaquine has been reported as yet, it is known that radical cure of vivax malaria caused by the Chesson strain requires increased doses of primaquine. Incidentally, the area of distribution of *P. vivax* Chesson strain contains the areas from which chloroquine-resistance of *P. vivax* has been reported. A recent report from Guatemala[50] points to a relatively low sensitivity of local *P. vivax* to primaquine. However, these infections responded to a higher total dose of the drug.

Formulations

Primaquine diphosphate is formulated as tablets containing 7.5 or 15 mg primaquine base (Neo-Quipenyl® or generic). The drug should be kept in air-tight containers and protected from light.

Dosage and Regimen

Primaquine is contraindicated in pregnancy and in children under 4 years of age. For radical cure of vivax malaria and ovale malaria in patients with normal G6PD levels primaquine is usually given at a daily dose of 0.25 mg/kg body weight for 14 days (total dose 3.5 mg/kg). In G6PD deficient patients the drug is usually tolerated without major haemolytic incidents when it is given on a weekly basis. For patients with low and moderate G6PD deficiency, weekly doses of 0.45 mg/kg. for 8 weeks are used. In patients with severe G6PD deficiency the weekly dose is 0.30 mg/kg for 15 weeks.

In areas where *P. vivax* Chesson strain occurs, a higher daily dose of primaquine or a longer duration of treatment is required, to bring the total cumulative dose to 6 mg/kg (distributed over 14-21 days). In G6PD deficient persons a weekly dose of 0.45 mg/kg can be used for 15 weeks (NB: in some areas affected by the Chesson strain the administration of radical treatment may not be warranted in the light of intensive transmission and high probability of re-infection).

For gametocytocidal treatment of falciparum malaria a single dose of 0.30 or 0.45 mg/kg is sufficient. This can be given without screening for G6PD status.

Side Effects and Adverse Reactions

At the doses used for anti-relapse treatment in vivax malaria and ovale malaria, primaquine is usually well tolerated by persons with normal G6PD levels. Anorexia, nausea, vomiting, abdominal pain and discoloration of urine may occasionally be observed.

In G6PD deficient persons intravascular haemolysis is the most important adverse effect. Its occurrence will depend on the dose and regimen of primaquine and the type of G6PD deficiency. Intravascular haemolysis is more likely to occur under a daily dose regimen than with weekly administration. Type B G6PD deficient persons are more prone to haemolysis than type A G6PD deficient people. Methaemoglobinaemia, manifesting with cyanosis, is usually associated with overdoses of primaquine. It is quite rare with antirelapse treatment, even at the elevated doses used for treating infections with the *P. vivax* Chesson strain.

Contraindications and Precautions

Primaquine is contraindicated in pregnant women and children < 4 years of age. These can be protected against relapses by weekly prophylaxis with chloroquine until such a time that treatment with primaquine becomes possible. If ever possible, the G6PD status of the patient should be assessed before starting treatment with primaquine in order to select the appropriate dose regimen. Even then, the treatment should be carried out under close supervision for an early detection of eventual intravascular haemolysis which would necessitate the immediate discontinuation of the drug. Concurrent treatment with other oxidant drugs, e.g. sulfonamides, may increase the risk of intravascular haemolysis and should therefore be avoided. Also the concurrent use of other,

non-oxidant, drugs requires close monitoring of the patient.

Antifolates

The term "antifolates" denotes drugs which interfere with enzymes involved in folate biosynthesis, i.e. a part of pyrimidine biosynthesis. Some are inhibitors of dihydrofolate reductase (DHFR), e.g. pyrimethamine, proguanil and its main metabolite cycloguanil. Others inhibit dihydropteroate synthase (DHPS), e.g. sulfonamides and sulfones. Members of the different groups are known to exert synergistic effects, e.g. sulfonamides with pyrimethamine, or sulfones with cycloguanil.

A) DHFR inhibitors

Pyrimethamine

Chemistry

Pyrimethamine is 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine (figure 11), a white microcrystalline substance that is obtained by chemical synthesis. It has a molecular weight of 248.7, is practically insoluble In water and sparingly soluble in ethanol. Pyrimethamine is nearly odourless and tasteless.

History

Pyrimethamine emanated from the major chemotherapeutic research programme conducted in U.K. and USA during World War II. It came into wider use in the late 1940s and was mainly employed for malaria prophylaxis and, for the purpose of sporontocidal action, as a complement to blood schizontocidal treatment. Due to resistance the use of pyrimethamine was very much reduced in the late 1960s. However, after the discovery of the potentiating effect of sulfonamides, overcoming moderate degrees of pyrimethamine resistance, the drug became popular again in the form of fixed combinations with suitable partners.

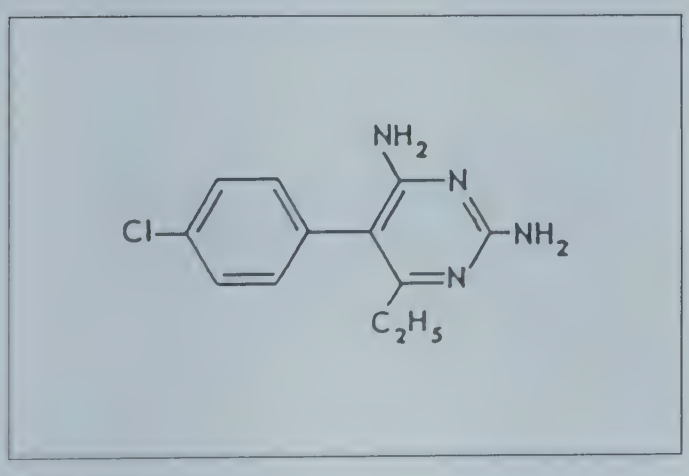

Fig. 11. Pyrimethamine.

Activity in Human Malaria

Pyrimethamine has blood schizontocidal activity in all species of human-pathogenic plasmodia, but this activity has been seriously compromised by resistance of *P. falciparum* and *P. vivax* soon after the introduction of the drug, especially in areas where it was used for mass suppression, e.g. in the form of medicated salt[16]. An alleged activity of pyrimethamine against pre-erythrocytic schizonts has never been substantiated. The same applies to gametocytocidal and sporontocidal activity in *P. falciparum*. In contrast to the 4-aminoquinolines and the 4-quinolinemethanols the blood schizontocidal activity of pyrimethamine is pronounced at the schizont rather than the trophozoite stage. This is also morphologically recognized by the formation of grossly abnormal schizonts with splintered chromatin.

Pharmacology and Pharmacokinetics

The blood schizontocidal effect of pyrimethamine is ascribed to the inhibition of DHFR (EC 1.5.1.4) The drug's affinity to this enzyme varies widely according to the species of pathogen and host. The affinity of pyrimethamine to plasmodial DHFR is known to be more than a thousand times higher than that to DHFR in the rat liver[51]. This explains differential toxicity/efficacy. Following oral administration pyrimethamine is well absorbed and C_{max} is reached within 2-6 hours (mean 0.94 ± 0.08 μmol.l^{-1} after an oral dose of 25 mg)[52]. There is little presystemic metabolism. The distribution is relatively narrow with a V_d of 2.9 l.kg^{-1} although in some tissues the drug is found in concentrations higher than in blood plasma. Binding to plasma proteins is high (87%). Plasma half-life varies between 35 and 175 hours, with a mean of

85 hours. Within 40 days approximately 1/4 of the drug is eliminated in the urine. Hepatic metabolism is extensive, with pyrimethamine-N-oxide as the main metabolite. There is no information on the biological activity of this metabolite. The pyrimethamine concentration in normal erythrocytes is approximately half the concentration in blood plasma. There is apparently no selective uptake of pyrimethamine by infected erythrocytes. Excretion of pyrimethamine with breast milk is minimal.

Resistance

Pyrimethamine was originally used as an agent for individual and mass prophylaxis, but infections with *P. falciparum* and *P. vivax* began to break through within 1-2 years of massive deployment of the drug, indicating resistance. Such resistance occurred in many parts of the area of distribution of the two species and practically ended the usefulness of monoprophylaxis with pyrimethamine.

After the discovery of the potentiating effect of sulfonamides and sulfones, enhancing the activity of pyrimethamine approximately 50-100 times and thus overcoming moderate degrees of resistance to this drug, the use of pyrimethamine was resumed in the 1970s, but mainly in the therapeutic context as an alternative medication in chloroquine-resistant falciparum malaria. In the 1980s resistance of *P. falciparum* to sulfonamide/pyrimethamine combinations became a problem in South America and parts of South East Asia, and it is increasing now in tropical Africa.

Formulations

Pyrimethamine alone is formulated as tablets containing 25 mg (Daraprim®, Erbaprelina®, Malocide®). These are used in the treatment of toxoplasmosis and other opportunistic protozoan infections, but nowadays rarely in the context of malaria.

Dosage and Regimen, Side Effects and Adverse Reactions, Contraindications

Since pyrimethamine is not anymore used as a mono-substance for the treatment or prophylaxis of malaria, these aspects are covered in the sections on combinations.

Proguanil

Chemistry

Proguanil is 1-(4-chlorophenyl)-5-isopropylbiguanide hydrochloride (figure 12). It is obtained by chemical synthesis. The free base has a molecular weight of 253.7, the hydrochloride a molecular weight of 290.2. By definition proguanil is a prodrug since its activity relies mainly on that of its main metabolite cycloguanil. Proguanil is a white, crystalline powder that is moderately soluble in ethanol and water. It has a bitter taste.

History

Like pyrimethamine, proguanil originated from the chemotherapeutic research programme conducted in U.K. and USA during World War II, and was commercially introduced in the late 1944s. Exploration of its therapeutic potential was given up soon after introduction since the 4-aminoquinolines had found their place in this domain, and proguanil was from then on only used for malaria prophylaxis.

Activity in Human Malaria

Proguanil by itself has weak blood schizontocidal and tissue schizontocidal activity in all human-pathogenic plasmodia. Its main metabolite, cycloguanil, shows both types of activity to a much higher degree. Neither proguanil nor cycloguanil act against hypnozoites. Little is known about an eventual gametocytocidal activity.

Although proguanil and cycloguanil are DHFR inhibitors, their interaction with the

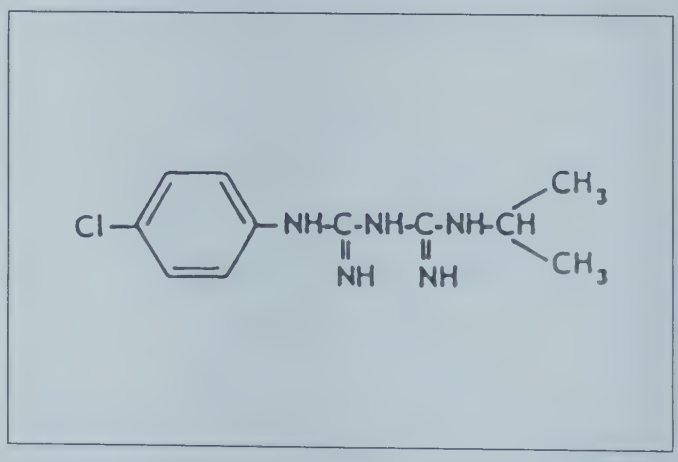

Fig. 12. Proguanil.

enzyme seems to differ from that of pyrimethamine[53], explaining differences in the prophylactic performance of the drugs.

Pharmacology and Pharmacokinetics

Apart from the specific antimalarial activity of proguanil and its main metabolite, cycloguanil, no other pharmacological effects of the compounds were reported. Proguanil is considered one of the safest antimalarial drugs. After oral administration proguanil is rapidly absorbed, becoming detectable in plasma within 15 minutes (in fasting subjects) and reaching C_{max} within 2-4 hours. There is little pre-systemic metabolism of proguanil. The mean plasma half-life of proguanil is 16.5 ± 3.5 hours. In plasma approximately 75% of the drug is bound to proteins. At an adult dosage of 100 mg per day steady state is reached within 4 days with peak levels of 647 ± 73 nmol.l^{-1} and trough levels of 228 ± 25 nmol.l^{-1}. With the currently practised daily adult dose of 200 mg steady state occurs within 3 days with mean C_{max} = 1258 ± 130 nmol.l^{-1} and a mean trough concentration of 354 + 53 nmol.l^{-1} [54].

Proguanil is metabolized to cycloguanil [4,6-diamino-1-(p-chlorophenyl)-1,2dihydro-2,2-dimethyl-s-triazine] (figure 13) which has higher biological activity as compared to the parent compound. The metabolic transformation is effected by a member of the coenzyme P450 group, apparently belonging to the P450 IIC family[55].

Metabolic transformation to cycloguanil shows individual variation and in normal subjects accounts for approximately 19% of the parent compound. However, there are poor metabolizers and these may fail to derive adequate prophylactic protection from proguanil. Cycloguanil usually becomes detectable within 2 hours from the ingestion of proguanil, reaching C_{max} within 4-9 hours. The mean half-life of elimination is 2.1 hours, explaining that the cycloguanil concentrations stay below those of proguanil. With a daily dose of 100 mg or 200 mg steady state concentrations of cycloguanil are reached within 4 days, with a mean C_{max} of 186 ± 30 nmol.l^{-1} and 293 ± 45 nmol.l^{-1} respectively. The difference between the through levels was less marked, with 115 ± 22 nmol.l^{-1} and 142 ± 37 nmol.l^{-1}. When the 200 mg dose was divided and 100 mg doses were given at 12-hourly intervals the cycloguanil profile was improved, with mean Cmax and mean trough levels of 317 ± 44 nmol.l^{-1} and 231 ± 35 nmol.l^{-1} respectively[54].

Besides cycloguanil another metabolite, 4-chlorophenylbiguanide, is formed which seems to lack antimalarial activity. Approximately 60% of proguanil is excreted unchanged in the urine.

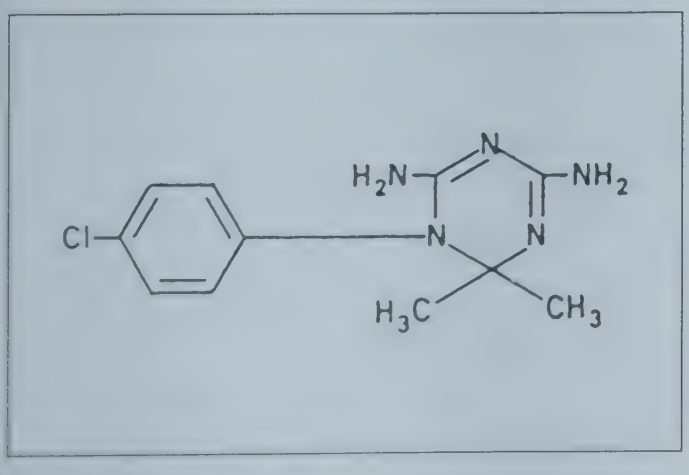

Fig. 13. Cycloguanil.

Resistance

In the absence of suitable test systems it is practically impossible to provide reliable information on the distribution of true resistance to proguanil/cycloguanil. There can be no doubt that the efficacy of proguanil is compromised in the hard-core areas of resistance to sulfonamide/pyrimethamine. However, a large proportion of proguanil failures reported in the literature is obviously due to an inappropriate dose regimen or a poor metabolizer status.

Formulations

Proguanil is formulated in tablets containing 100 mg proguanil hydrochloride (Bigumal®, Chlorguanide®, Diguanyl®, Paludrine®).

Dosage and Regimen

Proguanil is only used for the chemoprophylaxis of malaria, either alone or in association with chloroquine. On pharmacokinetic grounds the widely recommended and generally practised adult dose of 200 mg daily is unsound and certainly a reason of failure. The correct dose regimen for adults is 100 mg proguanil at in-

tervals of approximately 12 hours (a variation between 10 and 14 hours is acceptable), starting one week before exposure to malaria risk and continuing for 4 weeks after exposure has ended. The children dose is 2 mg/kg body weight at intervals of 12 (10-14) hours.

Side Effects and Adverse Reactions

Proguanil is a generally very well tolerated drug. Mild epigastric discomfort is the most frequent, albeit relatively rare, side effect. Mouth ulcers and stomatitis may occur. The frequency of these fully reversible adverse reactions is low, but apparently increases under simultaneous prophylaxis with chloroquine.

In persons with seriously impaired renal function the elimination of proguanil and cycloguanil may be compromised, giving rise to bone marrow suppression and haemopoietic disturbances.

In folate-deficient persons the symptoms of the deficiency may be aggravated by the use of proguanil. This can be prevented by a replenishment of the folate stores.

Contraindications and Precautions

Proguanil is contraindicated in persons suffering from impaired renal function. There are no other contraindications. Proguanil was found to be quite safe in pregnant women and in infants.

In persons known or suspected to suffer from folate deficiency the folate stores should be replenished before starting prophylaxis with proguanil.

Complementary Note

Chlorproguanil [1-(3,4-dichlorophenyl)-5-isopropylbiguanide hydrochloride], is a proguanil analogue with an elimination half-life that would ensure effective drug concentrations with one daily dose. However, the drug (Lapudrine®) was withdrawn, apparently for commercial reasons, before its advantageous pharmacokinetic parameters became known.

The antimalarial activity of proguanil and cycloguanil is known to be potentiated by sulfonamides and sulfones. However, this phenomenon has not yet been explored to any significant extent although it offers distinct promise.

B) DHPS inhibitors

Sulfadoxine

Chemistry

Sulfadoxine is N1-(5,6-dimethoxy-4-pyrimidinyl) sulfanilamide (figure 14). It is obtained by chemical synthesis and has a molecular weight of 310.3. Sulfadoxine is a white crystalline substance which is poorly soluble in ethanol and water. It is odourless and practically tasteless.

History

Sulfadoxine (sulformethoxine), a long-acting sulfonamide, has been developed in the 1950s as an antimicrobial agent. This was part of the effort of improving the original structure, sulfanilamide, with regard to antimicrobial spectrum and pharmacokinetic properties. When the antimalarial activity of the sulfonamides became better known it was realized that this group of compounds potentiates the effect of DHFR inhibitors. As pyrimethamine has a half-life similar to that of sulfadoxine, both compounds have been combined. The resulting combination of sulfadoxine/pyrimethamine subsequently became the most important replacement drug for the treatment of chloroquine-resistant falciparum malaria. For some time it was also employed for chemoprophylaxis, but this use has been discontinued in view of the risk of serious adverse reactions to the sulfonamide component.

Activity in Human Malaria

Sulfadoxine has moderate blood sch-

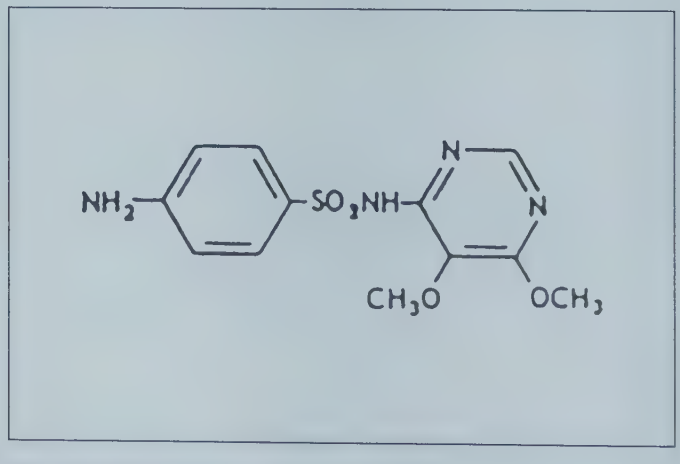

Fig. 14. Sulfadoxine.

izontocidal activity in *P. falciparum*. The same effect, but yet less marked, is seen in *P. vivax*. The antimalarial activity of sulfadoxine is not strong enough for using it in monotherapy. However, it enhances the activity of pyrimethamine approximately 50-100 fold. The exact mechanism of this synergism is not known, but it may be due to the interference with folate biosynthesis at two or more sites. Sulfadoxine has no activity on malaria parasite stages other than asexual blood forms where it interferes with schizont formation rather than with trophozoite growth.

Pharmacology and Pharmacokiratics

Sulfadoxine inhibits DHPS and interferes, therefore, with the incorporation of para-aminobenzoic acid (PABA) into folic acid. This effect is marked in all bacteria and protozoa which have to produce their own folic acid, but not in mammalian cells as these have to rely on the supply of intact folic acid.

The use of sulfadoxine as an antibacterial agent has been considerably reduced due to the development of specific resistance to this agent. However, in association with pyrimethamine it remains an important medicament in the treatment of malaria, toxoplasmosis and various opportunistic protozoan infections. Following oral administration sulfadoxine is well and rapidly absorbed and becomes detectable in blood plasma within 15 minutes, reaching C_{max} within 2-4 hours, with a mean plasma concentration of approximately 500 $\mu mol.l^{-1}$ after a dose of 1.6 g.

Approximately 94% of sulfadoxine in the plasma is bound to proteins. The volume of distribution is very narrow with 0.13 $l.kg^{-1}$. The plasma half-life is 150-200 hours. The long half-life of sulfadoxine can be ascribed to intensive enterohepatic circulation and to tubular reabsorption in the kidneys. Metabolic transformation of sulfadoxine is quite limited (< 10%). The metabolites are excreted in the urine.

Resistance

See section on combinations.

Formulations

Sulfadoxine is available in the form of tablets containing 500 mg (Fanasil®, Fanasulf®, Fanzil®) and as a solution containing 1 g in 4 ml (Fanasil®) for intramuscular injection. However, these formulations are largely irrelevant for the treatment of malaria since, in this context, sulfadoxine is used in association with pyrimethamine.

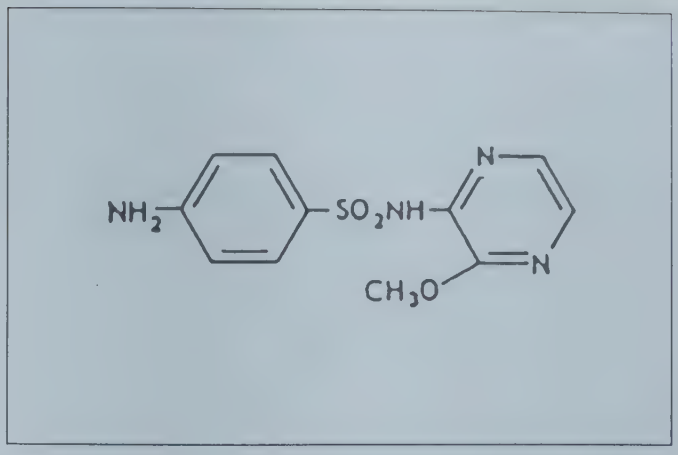

Fig. 15. Sulfalene.

Dosage and Regimen, Side Effects and Adverse Reactions, Contraindications

See section on combinations.

Sulfalene

Sulfalene is N'-(3-methoxy-2-pyrazinyl)-sulfanilamide (figure 15). Its antimalarial activity is practically identical with that of sulfadoxine. Its mean half-life is 65 hours, i.e. shorter than that of sulfadoxine and probably not in all cases able to match that of its partner drug pyrimethamine. However, the shorter half-life may express itself in a reduced severity of serious adverse reactions which are a problem with sulfadoxine. Sulfalene is available in the form of tablets containing 500 mg (Kelfizina®, Kelfizine®), but in the treatment of malaria it is used as a fixed combination with pyrimethamine.

C) Sulfones

Dapsone

Chemistry

Dapsone is 4,4'-diaminodiphenylsulfone, a synthetic compound with a molecular weight of 248.3. It is soluble in ethanol, but nearly insoluble in water. It is

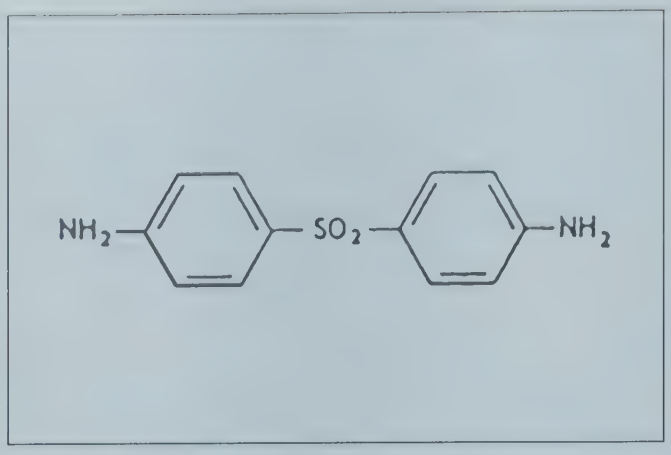

Fig. 16. Dapsone.

a yellowish-white. odourless crystalline powder (figure 16).

History

Dapsone was first synthesized in 1908. Its antibacterial properties were recognized in 1937 when it was investigated alongside the sulfonamides. Although highly effective it was considered too toxic for use in humans. Only later was it realized that the dose level was too high[56]. Subsequently dapsone and some of its analogues were found to possess mycobacteriostatic activity. By the end of the 1940s its value in the treatment of leprosy was firmly established. The observation that, in malarious areas, leprosy patients under dapsone treatment suffered less from malaria than the general population is said to have initiated the development of the synergistic combination of dapsone and pyrimethamine for malaria prophylaxis[56].

Activity in Human Malaria

Dapsone on its own exerts moderate blood schizontocidal activity against *P. falciparum* and less so against *P. vivax.* It is an inhibitor of DHPS and potentiates the effect of DHFR inhibitors, e.g. pyrimethamine. Its activity thus corresponds to that of the sulfonamides. Dapsone has no activity against plasmodial development stages other than asexual erythrocytic forms where it interferes with normal schizont formation.

Pharmacology and Pharmacokinetics

The pharmacodynamic activity of dapsone is based on its inhibition of DHPS. This is largely beneficial in the treatment of leprosy and the prophylaxis of malaria, but may be disadvantageous due to toxic effects on the haemopoietic system.

After oral administration dapsone is well absorbed and becomes detectable in the plasma within 15-30 minutes, reaching C_{max} within 1-3 hours (C_{max} approximately 6-7 $\mu mol.l^{-1}$). It has a rather wide distribution and around 50% of plasma dapsone is bound to proteins. Its mean plasma half-life is between 25 and 30 hours. The drug is extensively (> 80%) metabolized in the liver, mainly by N-acetylation and N-oxidation. Although there is some enterohepatic circulation, dapsone and its metabolites are almost completely excreted in the urine. At the dose regimen used, together with pyrimethamine, in malaria prophylaxis a true steady state of dapsone will not be built up.

Resistance

In the absence of a suitable *in vitro* test system for assessing the sensitivity of *P. falciparum* to dapsone, and since the drug is only used for prophylaxis (in association with pyrimethamine), indirect evidence of resistance can only come from breakthrough under prophylaxis. Such reports have been remarkably rare and they relate to areas of high therapeutic resistance to the combination of sulfonamides and pyrimethamine.

Formulations

Dapsone is available in the form of tablets containing 25 mg, 50 mg or 100 mg (Avlosulfone®, Damitone®, Daphone®, Diaphenylsulfone®, Diatox®, Diphenason®, Eporal®, Sulfadione®), mainly for use in the treatment of leprosy. For malaria prophylaxis dapsone is available as a fixed combination with pyrimethamine.

Dosage and Regimen, Side Effects and Adverse Reactions, Contraindications

See section on combinations.

D) Combinations of DHFR/DHPS Inhbitors.

Sulfadoxine/Pyrimethamine (S/P)

This is a synergistic combination of two agents which interact in two different areas of folate biosynthesis. Like its con-

stituents S/P acts only against asexual erythrocytic forms of malaria parasites, especially *P. falciparum*. It has only limited efficacy against *P. vivax* Its blood schizontocidal activity is most marked at the schizont stage, i.e. relatively late in schizogony. In view of a significant incidence of serious adverse reactions associated with the protracted use of S/P (prophylaxis) the combination is now restricted to treatment only. A noteworthy post-treatment phenomenon is the increased formation of gametocytes in *P. falciparum* infections. The cause is unknown.

Pharmacology and Pharmacokinetics

S/P combines the specific pharmacodynamic properties of sulfadoxine and pyrimethamine in the sense of a potentiation of pyrimethamine by sulfadoxine. After oral administration of correctly formulated S/P tablets both pyrimethamine and sulfadoxine are rapidly and well absorbed, becoming detectable in the blood plasma within 15-30 minutes. There is no pharmacokinetic interaction between the two drugs so that the kinetic parameters are largely as described for the individual components.

Resistance

While S/P retains much of its efficacy against *P. falciparum* in southern Asia west of Myanmar, Central America and large areas of tropical Africa, both the frequency and the degree of resistance in South America and parts of South East Asia and adjacent Oceania are such that S/P had to be replaced in the routine treatment of falciparum malaria.

Formulations

S/P is mainly formulated for oral medication, The tablets contain 500 mg sulfadoxine and 25 mg pyrimethamine (Fansidar®, Falcidar® and generic). Important deficiencies of bioavailability, especially of pyrimethamine, were observed with some S/P products. However, brands produced under "Good Manufacturing Practice". (GMP) are quality-controlled and unlikely to have this deficiency.

There is also a formulation for deep intramuscular injection (Fansidar®). Each ampoule of 2.5 ml contains 500 mg sulfadoxine and 25 mg pyrimethamine.

Dosage and Regimen

S/P is used for treatment only, especially that of uncomplicated falciparum malaria in areas where *P. falciparum* is likely to be chloroquine-resistant. The medication is given as a single dose.

The adult dose (individuals of ≥ 60 kg body weight) is three tablets, corresponding to a total of 1500 mg sulfadoxine and 75 mg pyrimethamine. The dose in persons weighing < 60 kg is adjusted to 25 mg sulfadoxine/kg or roughly 1/2 tablet per 10 kg. The necessity of (deep) intramuscular injection will rarely arise (e.g. emergency treatment of severe or complicated falciparum malaria in persons with hypersensitivity to Cinchona alkaloids). The dose per kg will be the same as with the tablets, i.e. 25 mg sulfadoxine + 1.25 mg pyrimethamine per kg up to a maximum dose of 1500 mg sulfadoxine and 75 mg pyrimethamine.

Side Effects and Adverse Reactions

The therapeutic single dose administration is generally well tolerated. Anorexia, nausea and vomiting are occasionally observed as well as pruritus and transient rashes. These side effects disappear spontaneously. Rare, but dramatic and potentially life-threatening adverse effects are major dermatological manifestations such as exsudative erythema multiforme, Stevens-Johnson syndrome and Lyell syndrome. These are due to hypersensitivity to sulfadoxine. Haematological manifestations such as leukopenia, thrombocytopenia, megaloblastic anaemia, purpura and agranulocytosis are extremely rare after a single dose of S/P. The incidence of dermatological adverse reactions with S/P may be increased in the presence of other sulfonamides or chloroquine, and in the case of a repetition of S/P medication at short interval.

Contraindications and Precautions

S/P is strictly contraindicated in persons with known hypersensitivity to sulfonamides. The drug should also not be given to patients who had received S/P treatment less than 4 weeks before, and to patients who are currently under treatment with other DHFR and/or DHPS inhibitors (e.g. trimethoprim). S/P is contraindicated

in infants < 2 months for the risk of kernicterus. Although S/P was found to be safe in pregnancy its use in pregnant women, especially during the first trimester, should follow very strict indication. Alternative medication with suitable antimalarials is preferable. S/P should not be given during the last month before term in order to avoid the risk of kernicterus in the newborn. Caution is recommended in patients who had recently received chloroquine for treatment or suppression. These patients should be advised to avoid strong exposure to sunlight (for 4 weeks).

Sulfalene/Pyrimethamine (SL/P)

This combination is practically an analogue of S/P. Thus the contents of the previous section apply also to SL/P, including the dose regimen for oral medication. The drug is available in the form of tablets containing 500 mg sulfalene and 25 mg pyrimethamine (Metakelfin®).

Dapsone/Pyrimethamine (D/P)

Dapsone and pyrimethamine interfere with plasmodial folate biosynthesis in two different areas. There is marked potentiation of the activity of pyrimethamine in the presence of dapsone. The combination is only used for prophylaxis where it seems to protect equally well from clinical manifestations of infections with all human-pathogenic plasmodia. D/P acts against the asexual erythrocytic parasites. An activity against pre-erythrocytic schizonts in the sense of a prevention of blood infection has been alleged but not sufficiently substantiated.

Pharmacology and Pharmacokinetics

The pharmacodynamic affects of D/P, both desirable and undesirable ones, are due to the inhibition of DHFR and DHPS by the two components. After oral administration of the tablet formulation of D/P, both components are well absorbed. Their plasma profiles and pharmacokinetic parameters are the same as observed with the administration of the single components. Thus, there is no indication of pharmacokinetic interaction between dapsone and pyrimethamine. A remarkable incongruency exists between the undisputed efficacy of D/P and the pharmacokinetic properties of dapsone and pyrimethamine. Theoretically, the combination is ill-matched in view of the short half-life of dapsone in relation to that of pyrimethamine. A possible explanation may be the maintenance of synergistic activity in the presence of low concentrations of dapsone.

Resistance

There is no *in vivo* or *in vitro* test system for the routine assessment of the response to D/P. Moreover, systematic and sufficiently extensive prospective studies of the protective efficacy of D/P in travellers to specific regions are lacking. Therefore little is known about the areas where failure of D/P is to be expected. The combination has apparently maintained a relatively high degree of efficacy, especially in tropical Africa[57, 58]. However, laboratory studies suggest that D/P may not be effective enough in areas where *P. falciparum* shows a high level of resistance to pyrimethamine[59].

Formulations

There is only an oral formulation of D/P. It contains 12.5 mg pyrimethamine and 100 mg dapsone per tablet (Maloprim®).

Dosage and Regimen

D/P is only used for malaria prophylaxis. The adult dose is 1 tablet (100 mg dapsone + 12.5 mg pyrimethamine) once a week, starting one week before exposure and continuing for 4 weeks after exposure has ended. Children doses (as from 4 years) should be adjusted to 2 mg dapsone/kg and week.

Side Effects and Adverse Reactions

As the dose of dapsone and pyrimethamine is rather low in malaria prophylaxis with D/P, the immediate tolerability of the drug is good except in individuals with hypersensitivity to sulfones which manifests with pruritus, fever and dermatitis. These conditions may progress to exfoliative dermatitis if the drug is not immediately discontinued. The most serious and potentially fatal, yet rare, adverse reactions are leucopenia, granulocytopenia and agranulocytosis. The risk of such serious adverse reactions increases with dosage and duration of D/P use.

Contraindications and Precautions

Hypersensitivity to sulfones or sulfonamides is an absolute contraindication to the use of D/P. D/P should not be used during pregnancy and , as significant amounts of dapsone are excreted in the breast milk, also not during lactation. Children under 4 years should receive alternative chemoprophylaxis.

Some countries, e .g . U. K., have introduced restricted prescription for D/P in view of the risk of potentially life-threatening adverse reactions. However, the already low risk can be further reduced by regular haematological checking at 2-monthly intervals.

Antibiotics

Doxycycline

Chemistry

Doxycycline is 4-dimethylamino-1, 4, 4a, 5, 5a, 6, 11, 12a, - octahydro-3, 5, 10, 12, 12a pentahydroxy-6-methyl-1, 11 -dioxo-2-naphthacenecarboxamide (figure 17) and belongs to the tetracycline group. This semi-synthetic antibiotic is used in the form of a the hydrochloride hemiethanolate hemihydrate which has a molecular weight of 512.9. It is a yellow crystalline powder that is readily soluble in water and moderately well in ethanol.

History

Doxycycline was introduced in 1966 as a broad-spectrum antibacterial agent of the tetracycline class. It has pharmacokinetic advantages over tetracycline in terms of lesser dose and longer dose intervals, but it used to be considerably more expensive. Its use in malaria dates to the early 1980s when it was introduced as a therapeutic supplement to quinine. Since the early 1990s it is also used for short-term malaria prophylaxis in areas with multidrug resistant *P. falciparum*.

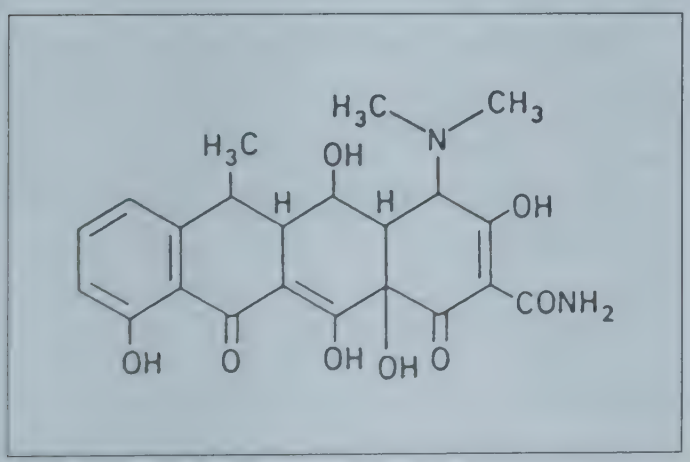

Fig. 17. Doxycycline.

Activity in Human Malaria

Doxycycline has tissue schizontocidal and blood schizontocidal activity against *P. falciparum* and probably the other human-pathogenic plasmodia. Its blood schizontocidal activity is too slow for being used for monotherapy in acute malaria, but in association with a fast acting, not necessarily radically curative, drug its effect is exhaustive resulting generally in a complete elimination of the blood infection. Doxycycline has no effect on hypnozoites and gametocytes. The antimalarial activity of doxycycline can be ascribed to the inhibition of protein synthesis and the chelation of Ca^{++}.

Pharmacology and Pharmacokinetics

The major pharmacological effect of doxycycline is directed against protein synthesis in microorganisms. Doxycycline is not mutagenic, but in utero it causes a retardation of skeletal development of the foetus. The same is observed in children throughout the major growth phases. Doxycycline may also cause photosensitivity. Following oral administration doxycycline is well absorbed (> 90%). C_{max} is usually reached within 3 hours, with 6-7 µmol < after an initial dose of 200 mg followed by daily doses of 100 mg. The trough levels are generally in the range of 3 -4 µmol/l. The distribution of doxycycline is rather narrow, with a V_d of 0.9-1.8 $l.kg^{-1}$ and most of the drug is bound to plasma protein (> 80%). The half-life of doxycycline is 18-22 hours. The drug is practically not metabolized and eliminated to nearly equal parts in the urine and in the faeces. Doxycycline is known to be excreted in the breast milk.

Resistance

While a considerable number of bacterial species have acquired resistance to

tetracyclines, including doxycycline, this does not seem to be the case with *P. falciparum*. In the absence of a suitable field method for *in vitro* testing it is not possible to perform quantitative monitoring of the sensitivity, but the follow-up of patients correctly treated with quinine and doxycycline does not indicate any loss of the clinical-parasitological efficacy of doxycycline. Similarly there is no report of failure with correctly performed prophylaxis using doxycycline.

Formulations

For oral administration doxycycline is formulated as capsules and tablets (Vibramycin®, Vibramycin-D®, Vibra-Tabs®, and generics) containing 50 mg or 100 mg of the drug. There are also formulations for use with intravenous, rate-controlled infusion, but-these are irrelevant in the context of malaria treatment since doxycycline is not employed in the context of the emergency management of severe and complicated malaria.

Dosage and Regimen

In the alternative treatment of falciparum malaria resistant to 4-aminoquinolines and sulfonamide/pyrimethamine combinations, doxycycline is used as a supplement to a fast acting drug that exerts rapid clinical and parasitological impact but by itself does not reliably produce radical cure, e.g. quinine.

Parallel to or following a course of quinine, doxycycline is given as a 7-day course. The regimen in adults consists of an initial loading dose of 200 mg followed by six doses of 100 mg at 24-hourly intervals. Children under 12 years of age and pregnant women should not be treated with doxycycline.

Doxycycline is also used for short-term prophylaxis in areas with multi-drug resistant *P. falciparum*. Here the potential risks due to the drug should be carefully weighed against the probability of infection before prescribing such prophylaxis to individuals who have no access to medical care while using the drug. For prophylaxis (starting two or three days before exposure) adults take an initial loading dose of 200 mg, followed by 100 mg daily, for a maximum duration of 6 weeks.

Side Effects and Adverse Reactions

Doxycycline is generally well tolerated. Nausea, anorexia and vomiting are rare. Potentially life-threatening adverse reactions may occur in the form of staphylococcal enterocolitis or pseudomembraneous colitis caused by *Clostridium difficile*. Photosensitivity may occur, especially in Caucasians. Maculopapular and erythematous rashes are occasionally seen, but exfoliative dermatitis and other hypersensitivity reactions are rare. Due to its antibacterial activity doxycycline may cause overgrowth of resistant microorganisms in specific locations and cause stomatitis, glossitis and vaginitis. Due to a suppression of normal gut bacteria it may also cause a reduction of vitamin K synthesis. In young individuals doxycycline may cause abnormal skeletal development, hypoplasia of enamel and permanent staining of teeth.

Contraindications and Precautions

Hypersensitivity to any member of the tetracycline group is a contraindication to the use of doxycycline. So is age below 12 years as well as pregnancy. Patients with systemic lupus erythematosus should not be given doxycycline since the tetracyclines are believed to exacerbate this condition.

As doxycycline is excreted in the breast milk, lactating women should not breastfeed their infants while under medication and until 4 days after completion of the treatment with doxycycline.

As there is a risk of phototoxic reactions, persons under therapeutic or prophylactic administration should be advised to avoid direct exposure to sunlight and to stop the drug at the first signs of erythema.

While under medication with doxycycline the plasma prothombin activity should be monitored in patients under therapy with anticoagulants and the dosage of the anticoagulants be adjusted accordingly.

Tetracycline

Chemistry

Tetracycline is 4-dimethyiamino-1, 4, 4a, 5, 5a, 6, 11,12a-octahydro- 3, 6, 10, 12,

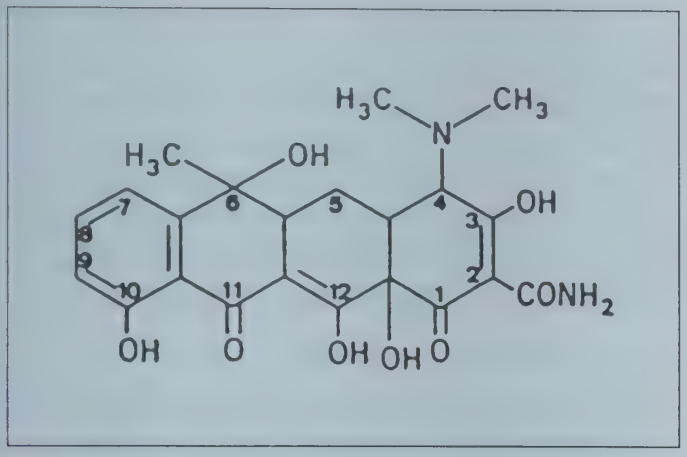

Fig. 18. Tetracycline.

12a pentahydroxy-6-methyl-1, 11-dioxo-2-naphthacenecarboxamide (figure 18), a semi-synthetic antibiotic produced from chlortetracycline. It is used in the form of a hydrochloride (molecular weight 480.9) that dissolves well in water, but sparingly in ethanol. It is a bitter-tasting yellow, odourless powder.

History

Tetracycline belongs to the early wide-spectrum antibiotics. It has been introduced in the 1950s and continues to be a useful drug in the treatment of various bacterial and rickettsial infections. Since the early 1980s it is being used, as a partner drug to quinine, in the treatment of falciparum malaria that is resistant to 4-aminoquinolines and sulfonamide/pyrimethamine combinations. Lately it has increasingly been replaced by doxycycline which is easier to use and now also cheaper (related to treatment cost) as compared to tetracycline.

Activity in Human Malaria

The same as doxycycline.

Pharmacology and Pharmacokinetics

The pharmadynamic effects of tetracycline are largely identical with those of doxycycline.

After oral administration tetracycline appears usually within 15-30 minutes in the blood plasma, and C_{max} is reached within 2-4 hours, with concentrations around 4 µmol/l at a dose of 250 mg. Absorption takes place mainly in the stomach and the small intestine, but it is incomplete and irregular, and influenced by the presence of food. Less than 50% of tetracycline in the plasma is protein-bound. The distribution is relatively narrow (V_d approximately 1.3 $l.kg^{-1}$). The approximate half-life is 8.5 hours, necessitating frequent dosing. There is very little metabolic transformation of tetracycline. Elimination is mainly by excretion with the faeces, with evidence of enterohepatic circulation. But a significant amount of tetracycline is also eliminated in the urine.

Resistance

Same as for doxycycline.

Formulations

Tetracycline for oral use is formulated as capsules or tablets containing 250 mg or 500 mg (Achromycin®, Achromycin V®, and generic). Tablets or capsules containing 250 mg are used in the treatment of malaria.

There are also formulations for the parenteral administration of tetracycline, but these have no application in the treatment of malaria.

Dose and Regimen

The onset of clinical improvement is too slow for using tetracycline for monotherapy in acute falciparum malaria. It is given as a supplement to a course of a fast-acting medicament, e.g. quinine. It may be given in parallel or subsequent to such a course. The adult dose of tetracycline is 250 mg (4 mg/kg) every 6 hours for 7 days. Due to the marked reduction of bioavailability by food incl. milk, the drug should be taken one hour before or two hours after meals. Tetracycline should not be given to children < 12 years or to pregnant women.

Tetracycline is not used in malaria prophylaxis.

Side Effects and Adverse Reactions

These are largely as described for doxycycline, but differences exist with regard to antimetabolic activity which is more marked in tetracycline. It will tend to manifest itself in patients suffering from impaired renal function with a cumulation of the drug, resulting in azotaemia, hyperphosphataemia and acidosis. Photosensitivity to tetracycline seems to be more frequent and marked than with doxycycline.

Contraindications and Precautions

These are essentially the same as mentioned for doxycycline. In addition, an increased frequency of life-threatening toxic reactions has been observed with the use of outdated tetracycline formulations which obviously contain toxic degradation products. The formation of such degradation products is likely to be accelerated by exposure to light and storage under moist and warm conditions. Proper storage of tetracycline and avoiding the use of outdated formulations are therefore important precautions against dangerous adverse reactions.

Clindamycin

Chemistry

Clindamycin is methyl-7-chloro-6, 7, 8-trideoxy-6-trans-(1-methyl-4-propyl-L-2pyrrolidinecarboxamido)-1-thio-L-threo-α D-galacto octopyranoside (figure 19). It is a derivative of lincomycin, and also known under the short name 7-(5)-chloro-7desoxylincomycin. It is formulated as a hydrochloride monohydrate with the molecular weight of 479.5. Clindamycin hydrochloride monohydrate is a white crystalline powder that is readily soluble in water and poorly soluble in ethanol.

History

Clindamycin has been introduced in the early 1970s as a broad-spectrum antibacterial agent. It was found to possess higher antimalarial activity as compared to lincomycin. It also has activity against several other protozoan parasites such as *Babesia microti* and *Toxoplasma gondii*. As an alternative antimalarial it has not become popular in endemic areas on account of high cost. As an antimalarial it is generally regarded as the medicament of last resort in the treatment of multidrug-resistant falciparum malaria.

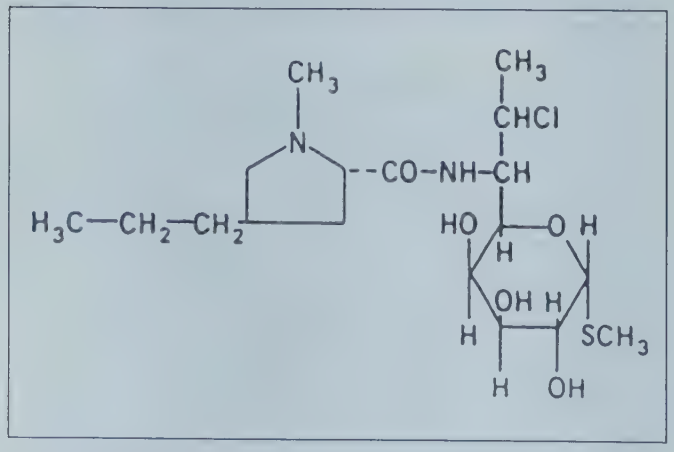

Fig. 19. Clindamycin.

Activity in Human Malaria

Clindamycin is known to have blood schizontocidal activity in *P. falciparum*. The same can be expected for *P. vivax* since the drug is also effective against *P. cynomolgi*. It is not known, whether clindamycin exerts tissue schizontocidal and/or gametocytocidal activity . Its antibacterial effect is based on the inhibition of nucleic acids, thereby interrupting protein synthesis. The mechanism of action in plasmodia seems to be the same.

Pharmacology and Pharmacokinetics

Clindamycin seems to lack mutagenic, teratogenic and carcinogenic activity. Besides its impact on microbial protein synthesis it appears to have no clinically relevant pharmacodynamic potential. Rapid intravenous administration of unusually high doses may lead to ventricular fibrillation and cardiac arrest.

Following oral administration clindamycin is extensively absorbed, reaching C_{max} within one hour. The drug is extensively bound to plasma proteins (> 90%). Despite a relatively wide distribution it is rapidly metabolized and eliminated, with a half life varying between 1.5 and 3.5 hours in adults. This may account for a relatively high variability of C_{max} levels (mean 6 µmol/l). Metabolism of clindamycin is mainly hepatic by N-demethylation, sulfoxidation and hydrolysis. Only about 10-15% of clindamycin is excreted in the form of the parent drug, one third in the faeces, and two thirds in the urine. There is apparently no risk of cumulation of clindamycin and its metabolites when the drug is given at 6-hourly intervals. Clindamycin crosses the placenta. It is also excreted at significant extent in the breast milk.

In contrast to the tetracyclines food intake does not appreciably reduce the bioavailability of clindamycin.

Resistance

There is so far no report of plasmodial resistance to clindamycin. This is also not expected since the drug is, also as an antibacterial agent, not widely used in malarious areas on account of its cost. Little is known about antimalarial activity correlations between clindamycin and the tetracyclines.

Formulations

For oral administration clindamycin hydrochloride is formulated as capsules containing 75 mg base, 150 mg base or 300 mg base (Clindamycin, Dalacin®, Sobelin®). There is also a granulate for the preparation of an oral suspension containing 15 mg/ml. Formulations for parenteral administration are irrelevant in the context of malaria treatment since clindamycin is not used in the emergency management of severe and complicated falciparum malaria.

Dose and Regimen

The only indication of clindamycin in the treatment of malaria are infections with multidrug resistant *P. falciparum*. Clinical improvement seems to be faster than with tetracyclines. Nevertheless it is usual to rely for rapid clinical improvement on a fast-acting drug, e.g. quinine, before giving clindamycin for radical cure. Parallel treatment with both drugs is not advisable as it is thought to increase the risk of pseudomembraneous colitis associated with clindamycin.

In the treatment of falciparum malaria the dose of clindamycin is 5 mg/kg at 6 hourly intervals for a duration of 7 days. Pregnant women and infants < 3 months should not be treated with clindamycin.

Side Effects and Adverse Reactions

Clindamycin is not a medicament of primary choice in the treatment of malaria since up to 20% of the patients may develop diarrhoea which, in some cases may give rise to pseudomembraneous colitis, a potentially fatal adverse reaction. Otherwise the drug is generally well tolerated, but rare cases of anaphylactic shock and Stevens-Johnson syndrome have been reported.

Contraindications and Precautions

Pregnant women and infants < 3 months should not be treated with clindamycin. The same applies to developmentally retarded infants < 1 year due to reduced renal function. As significant amounts of clindamycin are excreted in the breast milk lactating women should not breast-feed their infants while under treatment with the drug. Known hypersensitivity to clindamycin is an absolute contraindication to its use.

If diarrhoea occurs during the treatment with clindamycin the drug should be discontinued immediately. The diarrhoea may be orally treated with vancomycin which prevents the overgrowth of causative *Clostridium difficile*.

Artemisinin and its derivates

Chemistry

Artemisinin (qinghaosu) is a sesquiterpene lactone (figure 20) that occurs naturally in the annual plant *Artemisia annua* L. from which it can be extracted with organic solvents. Artemisinin has a molecular weight of 282.3 and is a white crystalline substance with very low solubility in water. It is readily soluble in absolute methanol and ethanol. As it was initially intended to use artemisinin parenterally and in view of its relatively poor solubility in injectable oils, several derivatives of artemisinin were produced of which artemether, sodium artesunate and dihydro-artemisinin have been commercially formulated. Artemether

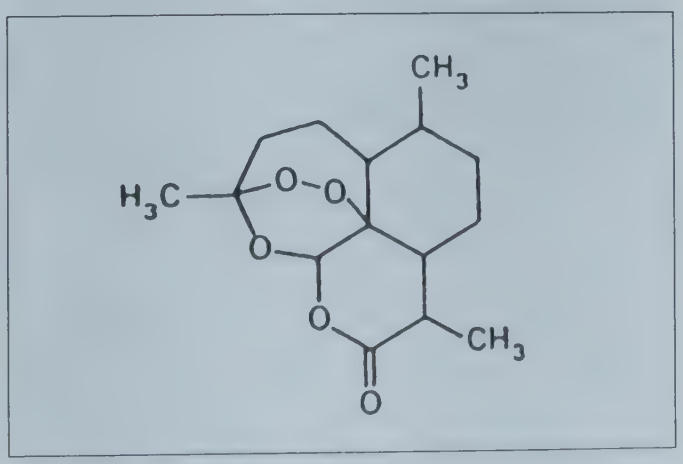

Fig. 20. Artemisinin.

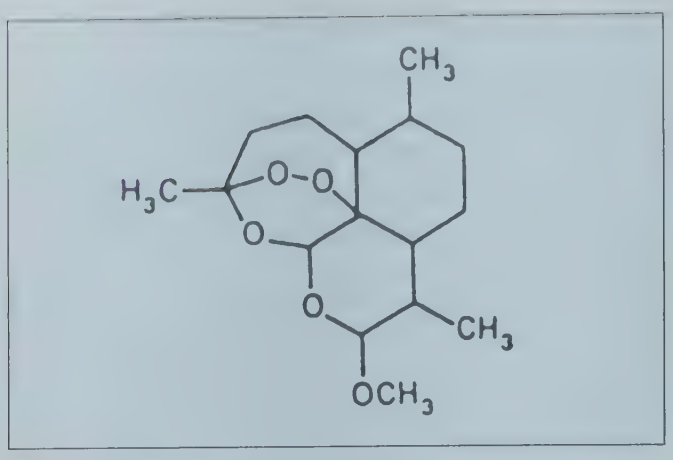

Fig. 21. Artemether.

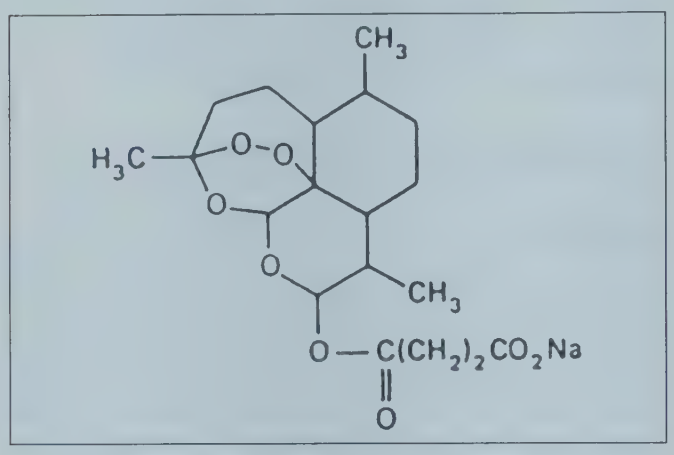

Fig. 22. Na-artesunate.

(figure 21) is the methyl ether of artemisinin. It is poorly soluble in water, but it dissolves well in injectable oils. Sodium artesunate (figure 22) is the Na-hemisuccinyl ester of artemisinin. It is readily soluble in water, but quickly hydrolyses under formation of dihydro-artemisinin which is also the main (active) metabolite of artemisinin. *De novo* chemical synthesis of artemisinin has been achieved[60], but it is unlikely to become a feasible alternative to the natural material, except in the production of labelled compounds for research purposes.

History

The ancient and recent history of artemisinin is full of errors and misinterpretations. The medicinal use of *Artemisia annua* (qinghao) was first mentioned in 168 BC as part of the "Recipes for 52 kinds of disease" which were discovered in the Mawangdui Han Tomb, Changsa, Hunan Province, China[3].

In these recipes it was described as a remedy against haemorrhoids. The first recorded use as an antimalarial dates to 340 AD during the Eastern Dynasty in China, when Ge Hong mentioned it in his Handbook of Prescriptions for Emergency Treatments[3]. In the way the plant material was used only minimal amounts of artemisinin could have been taken up by the patient, certainly not enough for specific antiplasmodial activity and the beneficial effect of the treatment is probably to be ascribed to the general antipyretic properties of many *Artemisia* species some of which were important components of European pharmacopoeas as early as the 17th century.

In 1972 Chinese scientists succeeded in extracting artemisinin from Artemisia annua material, mainly leaves and flower buds. It was accompanied by a number of other terpenes and precursors of artemisinin, but only artemisinin was found to possess marked antimalarial activity. The artemisinin content of the fresh plant material varies generally between 0.05 and 0.5% according to the origin of the plants, local soil conditions and climate.

Marked differences between the activity of orally and parenterally administered artemisinin were initially ascribed to an excessive first pass effect leading to an inactivation of a large part of the oral dose. This stimulated the formulation of artemisinin derivatives for parenteral use, e.g. artemether and Na-artesunate[61]. It was only later that these derivatives were found to be well absorbed via the gastrointestinal tract, without major loss by a first pass effect. These observations were recently extended to dihydro-artemisinin, the most effective artemisinin derivative and metabolite of the parent drug and all its pharmaceutic derivatives. The initial confusion could have been avoided had reliable and sufficiently sensitive assay methods been available.

Activity in Human Malaria

Artemisinin exerts rapid blood schizontocidal activity against all mammalian and avian plasmodia so far tested. On a molar

basis artemether is slightly more active than artemisinin against *P. falciparum in vitro*. Dihydro-artemisinin is the most active compound among the derivatives. The rapid action is also clinically evident and makes this class of antimalarials the fastest acting known to date. Circumstantial evidence points also to a gametocytocidal effect in *P. falciparum*. Artemisinin and its derivatives have no activity against pre-erythrocytic schizonts and hypnozoites. They also seem to lack sporontocidal activity.

The mechanism of action of artemisinin consists of two stages, namely activation by intra-parasitic, iron-catalysed generation of free oxygen radicals. followed by alkylation and bonding with parasite proteins[62]. In vitro in *P. falciparum* the activity of artemisinin resembles that of disinfectants rather than that of enzyme blockers[33]. Electron microscopic morphological observations point in the same direction[63].

Pharmacology and Pharmacokinetics

The acute toxicity of artemisinin and its derivatives is quite low and at clinically relevant doses symptomatic side effects are rare. The only well investigated pharmacodynamic effect relates to the antimalarial effect although it is conceivable that the selective activity against fast-dividing cells may offer a wider clinical spectrum, especially in association with other chemotherapeutic agents.

The exploration of the pharmacokinetics of artemisinin and its derivatives was for a long time beset by major technical constraints inasmuch as the UV spectra of artemisinin and most of its derivatives are in an area where co-extracted endogenous substances do not permit reliable and reproducible HPLC assays. All data emanating from such assays with or without prior derivatization can at best be considered semiquantitative approximations. The development of HPLC methods with electrochemical detection in the reductive mode[64] permits the reliable measurement of artemisinin, artemether, arteether and dihydro-artemisinin in biological fluids with reasonable limits of detection. Unfortunately these methods are quite demanding in time, dexterity and cost.

The pharmacokinetics of artemisinin and its derivatives show considerable differences depending on the compound and the mode of administration. The intravenous (bolus) injection of Na-artesunate produces immediately peak plasma levels of Na-artesunate and dihydro-artemisinin (60-120 μmol/l after a dose of 2-3.8 mg/kg body weight). After a short while only dihydro-artemisinin will be detectable since practically all Na-artesunate hydrolyses under formation of this compound. The plasma concentrations recede rapidly as the elimination half-life of dihydro-artemisinin is only approximately 45 minutes. Elimination is mainly urinary in the form of deoxy-metabolites. Due to lack of appropriate studies little is known about the pharmacokinetics of Na-artesunate after intramuscular injection although this mode of administration was found clinically equally effective as compared to i.v. administration[65].

Following the intramuscular injection of artemether (dissolved in peanut oil) C_{max} is usually observed within 4-9 hours, with artemether concentrations between 0.5 and 0.8 μmol/l at doses of 6-10 mg/kg. In view of the relatively slow release from the intramuscular depot there is no reliable information about half-life. After intramuscular injection part of the artemether is metabolized to dihydro-artemisinin, but as the half-life of this metabolite is rather short, the concentrations of dihydro-artemisinin tend to be very low.

When given orally, artemisinin becomes usually detectable in the blood within one hour, and peak levels are reached within 4-8 hours, with C_{max} values of 0.7-0.9 μmol/l after a dose of 5 mg/kg. This points to poor absorption. Due to lack of appropriate studies there are no reliable data on half-life and metabolism, but it seems that only a relatively small part of the systemically available artemisinin is converted to dihydro-artemisinin.

Orally administered artemether becomes detectable in the blood within 15-30 minutes, reaching C_{max} within 90-120 minutes. Dihydro-artemisinin appears in the plasma usually within 30 minutes reaching peak concentrations shortly after the C_{max} of artemether. The mean peak concentrations of artemether after an oral

dose of 3 mg/kg was approximately 1 µmol/l, with considerable inter-individual variation. The half-life of orally administered artemether is relatively short (120-150 minutes).

There is practically no information on the pharmacokinetics of orally administered Na-artesunate and dihydro-artemisinin although both drugs were found to be clinically effective after oral dosing.

Similarly there is only scanty information on the pharmacokinetics of artemisinin following rectal administration in the form of suppositories. Preliminary observations point to relatively poor absorption, a C_{max} of approximately 0.03 µmol/l 6 hours after drug administration (10 mg/kg) and a half-life of 4 hours.

Resistance

Artemisinin is a compound that bears no structural resemblance to any of the classical antimalarials from which it also differs in the mode of action. In the absence of specific drug pressure it would be therefore surprising to encounter primary resistance to artemisinin and its derivatives. All reports point so far to a satisfactory response of *P. falciparum*. However, the information is not complete since there were no studies in areas where artemisinin and its derivatives were massively and haphazardly used over the past years. This refers particularly to parts of the Indochina Sub-Continent.

In long-term *in vitro* culture under specific drug pressure it is possible to produce *P. falciparum* with reduced sensitivity to artemisinin. According to own recent experience with several *P. falciparum* populations isolated before treatment and after recrudescence this happens faster *in vivo* in the course of treatment failure.

Formulations

There is a plethora of formulations of artemisinin and its derivatives for oral, parenteral and rectal administration. Most of these are registered in their countries of origin in eastern Asia. but not yet internationally on a larger basis as most formulations do not yet meet the appropriate regulatory requirements.

Artemisinin for oral use is formulated in tablets or capsules containing 250 mg. There is also a suppository formulation containing 100 mg artemisinin, mainly for paediatric use. Most of the artemisinin formulations originate from Vietnam.

Artemether for oral use is formulated as capsules containing 40 mg and as tablets containing 25 mg or 50 mg of β-artemether. Artemether is also available as injectable solution in peanut oil for intramuscular administration. The ampoules contain 80 mg in 1 ml peanut oil.

Artesunic acid is formulated in tablets (50 mg) for oral use. For parenteral use it is available as an anhydrous powder (60 mg/ampoule), accompanied by ampoules containing 0.6 ml 5% (w/v) sodium bicarbonate solution. Immediately before use 60 mg artesunic acid are dissolved in 0.6 ml sodium bicarbonate solution. This is made up with 5.4 ml dextrose solution (5%). The resulting solution can be administered by intramuscular or intravenous injection (within 5 minutes from reconstitution). Use in rate-controlled intravenous infusions is strictly contra-indicated due to the instability of the solution.

Dihydro-artemisinin is formulated for oral use as tablets containing 50 mg or 60 mg.

Although being probably the best documented derivative of artemisinin its ethyl ether, arteether, is not yet registered with any formulation.

Dosage and Regimens

In the absence of adequate pharmacokinetic information all dose recommendations for artemisinin and its derivatives have to be considered preliminary. However, there are some general rules on dose limitations the observation of which should avoid potentially dangerous adverse reactions.

The total adult dose of oral artemisinin should not exceed 2500 mg (40 mg/kg), that of oral artemether, artesunic acid, or dihydro-artemisinin should be limited to 600 mg (10 mg/kg). The same applies to parenteral total doses of Na-artesunate. With artemether by the intramuscular route a total dose of 320 mg (~ 6 mg/kg) should not be exceeded in view of the risk of neurological adverse reactions.

At these total doses monotherapy with artemisinin and its derivatives will not be radically curative in a considerable num-

ber of cases. Supplementary medication with suitable antimalarials is therefore mandatory in order to avoid recrudescences and the inherent risk of promoting resistance.

The parenteral administration of artemisinin derivatives should be restricted to severe and complicated malaria. Na-artesunate by the intravenous or intramuscular route will be the drug of choice. The first dose is approximately 2 mg/kg body weight (120 mg for adults), followed by two doses of 1 mg/kg at 12-hourly intervals and then daily doses of 1 mg/kg until the patient is able to take oral medication. Total cumulative dose 10 mg/kg. This should be followed by supplementary radically curative medication. When artemether is used by the intramuscular route the first dose is 3.2 mg/kg, followed by two more doses of 1.6 mg/kg at 24-hourly intervals. Further oral treatment with 12-hourly doses of 1.6 mg/kg until a total cumulative dose of approximately 10 mg/kg, followed by radically curative supplementary medication.

Where parenteral drug administration is not possible and emergency treatment needs to be given to unconscious patients before admission to hospital, artemisinin suppositoria may be used (1 suppositorium for patients ≤ 20 kg; 2 suppositoria for patients of > 20 kg).

For the alternative treatment of multidrug resistant uncomplicated falciparum malaria artemisinin and its derivatives are always given with a supplementary drug in order to ensure radical cure and to avoid the risk of promoting drug resistance. In the interest of compliance the regimens should be completed within two days. A typical example is the treatment with artesunate or artemether supplemented by mefloquine:

Day 0 Artemether or Artesunate 2 m/kg
Day 1 Artemether or Artesunate 1 mg/kg + Mefloquine 15 mg/kg
Day 2 Artemether or Artesunate 1 mg/kg + Mefloquine 10mg/kg

The initial treatment with artemether or artesunate alone will rapidly improve the clinical condition and increase the tolerability of mefloquine.

Artemisinin and its derivatives are the fastest acting antimalarial drugs known to date. Fever clearance and parasite clearance are usually achieved within 36 hours, i.e. at a time when only a relatively small total dose has as yet been taken.

Side Effects and Adverse Reactions

At the indicated therapeutic dose levels artemisinin and its derivatives are generally very well tolerated. Transient drug-induced fever seems to be the most frequent adverse effect (up to 25%), while transient first-degree heart block appears to be a rare event[66]. Haematological adverse reactions such as granulocytopenia or a reduction of reticulocytes are dose-dependent phenomena which rarely occur with normally dosed treatment

Neurotoxicity observed in rats and dogs given artemether or arteether i.m.[67] gave rise to alarm with regard to the safety of these drugs. However, both drugs were given at doses and over times largely exceeding those used therapeutically in man. As this phenomenon was not observed with the oral administration of artemether in comparable doses it may be due to the cumulation of the drug and/or its metabolites. This remains unclear in the absence of pharmacokinetic data. It would nevertheless be wise to limit the intramuscular administration of oily solutions of artemisinin derivatives to a total cumulative dose of 6.4 mg/kg.

Contraindications and Precautions

Hypersensitivity to artemisinin and its derivatives is an absolute contraindication to the use of these drugs

Artemisinin and its derivatives are known to be foetotoxic during the early stages of pregnancy, even at doses close to those used in treatment. Therefore these drugs are contraindicated in early pregnancy. Although the infants born to mothers treated with artemisinin or its derivatives after 15 weeks of gestation showed no abnormalities and the drugs are known to lack mutagenic or teratogenic activity[68] it would be wise to restrict their use during the second and third trimester of pregnancy to cases of vital indication.

Advanced candidate drugs

Pyronaridine

Pyronaridine has been synthesized by

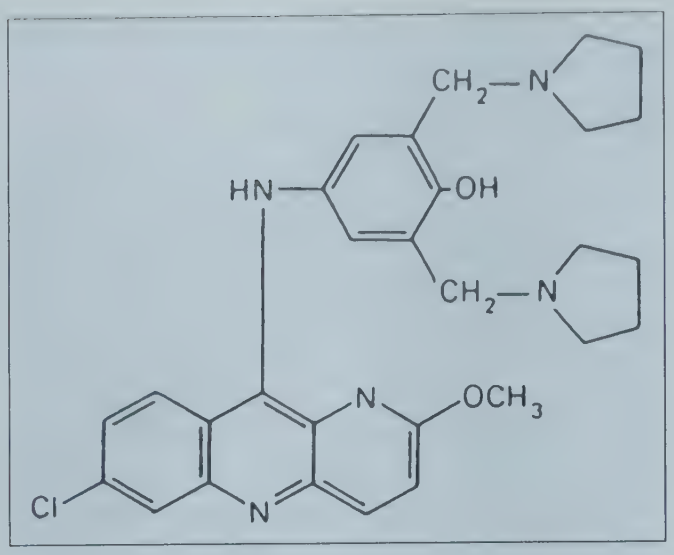

Fig. 23. Pyronaridine.

Chinese scientists in 1970[69] and found to possess marked antimalarial activity in rodent, simian and human malaria[70]. Chemically it is 2-methoxy-6-chloro-9 [3,5-bis(1-pyrrolidinylmethyl)-4-hydroxy] anilino-1-azaacridine (figure 23) and belongs to the Mannich bases. It is formulated as a tetraphosphate which crystallizes in yellow needles. The tetraphosphate is readily so!uble in water. The aqueous solution is intensily red.

The antimalarial activity of pyronaridine is blood schizontocidal in all *Plasmodium* species so far tested. There is also gametocytocidal activity in all species except *P. falciparum* and probably also *P. reichenowi*. Pyronaridine appears to exert tissue schizontocidal activity in rodent malaria[71], but appropriate studies in human malaria are lacking. There is apparently no hypnozoitocidal or sporontocidal activity. The mechanism of action is largely unknown but presumed to be directed against the parasite's protein metabolism. The blood schizontocidal activity of pyronaridine is correlated with that of chloroquine[72, 73], but as the relative efficacy of pyronaridine is much higher the drug is also effective in chloroquine-resistant falciparum malaria. However, resistance to pyronaridine can be rapidly induced in animal models and in *P. falciparum in vitro*.

Sufficiently sensitive and reliable assay methods for pyronaridine in biological fluids have only very recently been developed. Thus there is practically no information as yet on the pharmacokinetics. Such information is all the more important as there seems to be a problem with the bioavailability of orally administered pyronaridine which could explain the variability of clinical-parasitological response in falciparum malaria[74]. Appropriate pharmacokinetic studies are currently being undertaken.

Although available toxicological data and experience in the treatment of falciparum and vivax malaria suggest good tolerability it has to be realized that the pre-clinical dossier of pyronaridine is far from complete. This relates particularly to mutagenicity, teratogenicity and carcinogenicity studies and the potential occurrence of hypersensitivity reactions.

Pyronaridine is certainly a candidate drug meriting further development. The first priority concerns the completion of the pre-clinical data base, the second the resolution of the obvious problem of bioavailability associated with the oral administration of pyronaridine. The ease with which plasmodial resistance can be induced further suggests that monotherapy with pyronaridine may not be the ideal way of using this drug once its safety has been firmly established. This requires the systematic exploration of suitable partner drugs to be used in association with pyronaridine.

Atovaquone

The antimalarial activity of certain naphthoquinones is known since the 1940s, but their development was discontinued mainly for reasons of low and variable bioavailability when given by the oral route. Renewed interest in this chemical group led to the synthesis of a new analogue, BW 58C, which showed blood and tissue schizontocidal activity in various animal malaria models and had reached the stage of clinical Phase-I trials. It was superseded by compound 566 C80, atovaquone, which was found to possess improved pharmacodynamic and pharmacokinetic characteristics. Atovaquone is 2-[trans-4-(4'-chlorophenyi) cyclohexyl]-3-hydroxy-1,4-naphthoquinone (figure 24). The compound was found to act also against several protozoan parasite

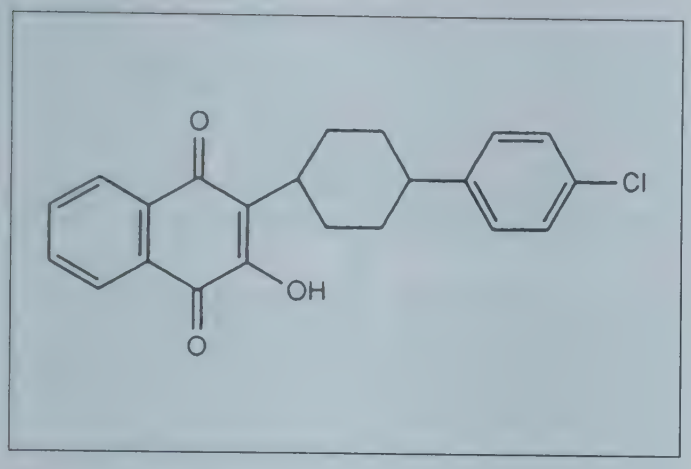

Fig. 24. Atovaquone.

species responsible for opportunistic infections associated with AIDS such as *Pneumocystis carinii* and *Toxoplasma gondii*[75]. Although originally developed for antimalarial use, it has been registered for the treatment of AIDS-associated infections.

Atovaquone has blood schizontocidal and tissue schizontocidal activity in rodent and primate plasmodia[76, 77]. Its mechanism of action appears to be based on the inhibition of mitochondrial respiration at the cytochrome bc1 complex[78]. Its mechanism of action appears to be different from that of the antimalarials in current use. However, *in vitro* investigations in fresh African isolates of *P. falciparum* indicate a relatively wide inter-individual variation of response with EC_{99} values above the concentrations likely to be used with therapeutic doses of atovaquone[79]. This would, in part, explain the relatively high failure rate with atovaquone when used in monotherapy for the treatment of multidrug-resistant falciparum malaria[80].

An additional explanation of the relatively high clinical-parasitological failure rate may be sought in the variable bioavailability of the compound which seems to be improved with food intake. This is certainly a field of further exploration including galenic aspects.

The results in the treatment of falciparum were improved by the combined use of atovaquone and proguanil[80], and biguanides seem to be the most suitable partners to atovaquone. This would also make sense when the prophylactic use of such a combination is contemplated since both drugs exert tissue schizontocidal activity. However, in view of the long half-life of atovaquone of approximately 70 hours[76] and the considerably shorter one of proguanil the combination does not appear to be ideal. This could be improved by the use of chlorproguanil the half-life of which is closer to that of atovaquone.

Outlook

Over the past decade support of research towards the development of new antimalarials has been significantly reduced, both in governmental institutions and in the pharmaceutical industry. The decreased funding in governmental institutions is in part due to financial constraints related to economic crisis, to another part due to the resolution of the East-West conflict that has also curbed the risk of major warfare in the tropics. Industry has been increasingly opting out from antimalarial research due to exponentially rising cost of the development of drugs. Medicaments for affluent markets are more likely to recover research and development investment, and to yield a handsome profit, but drugs for shaky and in addition often regulated markets are considered a doubtful investment. Nevertheless a number of promising candidate compounds are in the pipeline. These compounds originate almost exclusively from governmental research institutions which, by themselves, are not in the financial and technical position of sustaining the development to registration. Thus there is a risk that some of the promising leads may not come to realization. This is particularly to be expected with drugs for a rather limited indication, e.g. hypnozoitocidal compounds.

The new leads may be classified into the exploration of compounds belonging to chemical classes which are already employed as antimalarials and into the investigation of compounds belonging to new chemical classes. A third category are compounds which, although devoid of antimalarial activity, may enhance the parasite's response to classical drugs and help overcoming specific resistance.

Among the compounds belonging to

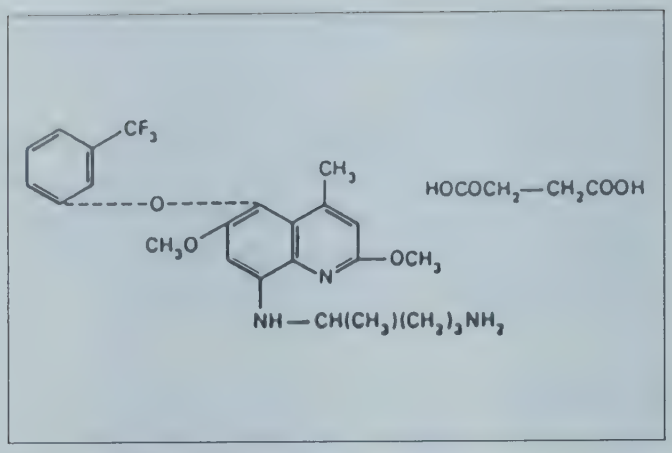

Fig. 25. WR 238,605.

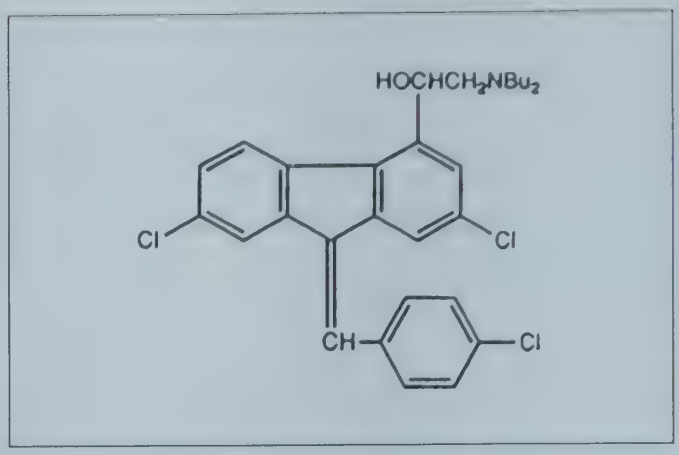

Fig. 26. Benflumetol.

classes known for their antimalarial activity, WR 238,605, a 5-phenoxy-substituted 8-aminoquinoline (figure 25), is probably the most advanced. Its tissue schizontocidal activity is much higher than that of primaquine[44]. It has also appreciable blood schizontocidal activity and seems to synergize with chloroquine in the sense of a reversal of resistance[81]. This compound has reached the clinical trial stage.

Another and as yet less advanced primaquine analogue, namely trifluoroacetylprimaquine oxalate (M8506) was found to be less toxic and as a hypnozoitocidal drug in the *P. cynomolgi/Macaca mulatta* model more effective than primaquine[82].

The exploration of a series of 2,3-N-substituted 4,4'-diaminophenyl sulfones showed that six members of this class had higher suppressive and curative activity in the *P.berghei*/mouse model and a lower toxicity as compared to dapsone[83]. This observation may be of particular value in the development of synergistic antifolate combinations.

Compound PS-15, a hydroxylamine-derived biguanide was found to be a potent inhibitor of DHFR, and in its activity independent from pyrimethamine and cycloguanil[84]. After oral administration it is partially metabolized to WR 99,210, a dihydrotriazine and highly active DHFR-inhibitor. Both PS-15 and WR 99,210 are blood schizontocides and exert also some tissue schizontocidal activity. In the *P.cynomolgi bastianellii/Macaca mulatta* model it only delays tissue schizogony and does not prevent it[85]. WR 99,210 had previously undergone extensive preclinical and clinical studies. This may accelerate the development of PS-15.

Realizing that the activity of artemisinin and its derivatives is based on the trioxane structure of ring B, containing a peroxide bridge, a considerable number of trioxanes were synthesized and investigated for their antimalarial activity[86, 87, 88]. These synthetic trioxanes are much simpler in their chemical structure than the natural model. Several compounds, especially tricyclic 1,2,4-trioxanes proved to be highly effective blood schizontocides in the *P. berghei*/mouse and the *Aotus/P. falciparum* models.

In an extension of the synthesis effort several Dispiro-1,2,4,5-tetraoxanes were produced and tested for their antimalarial properties[89], yielding evidence of low toxicity and activity equivalence with artemisinin.

Among the compounds with novel chemical structure, benflumetol is obviously the most advanced. Benflumetol is a fluorene (benzindene) derivative (figure 26) and has been synthetised at the Academy of Military Medical Sciences, Beijing[44]). It is well tolerated and has blood schizontocidal activity against all rodent, simian and human plasmodia so far tested. It is also effective against chloroquine-resistant *P. falciparum*. Used in monotherapy benflumetol has a relatively slow, but rather reliable activity. It is currently undergoing clinical trials in an oral combination with artemether.

Compound WR 182, 393, a guanylhy-

drazone, exhibited causal prophylactic and radical curative properties when tested in the *P. cynomolgi/Macaca mulatta* model[90]. This is so far the only compound outside the 8-aminoquinoline group that offers prospects for novel antirelapse drugs.

The modulation of chloroquine resistance of *P. falciparum* by verapamil and other calcium channel blockers *in vitro*[41, 42, 91] raised considerable hope with regard to the practical applicability of the principle, but clinical observations failed to show the same in the *in vivo* situation[43]. However, this chapter is not closed as yet since modulation may be achieved in different ways. Thus a novel pyrrolidino-alkane-amine compound (WR 268,954) with low intrinsic antimalarial activity was found to potentiate the activity of chloroquine and quinine in chloroquine-resistant isolates of *P. falciparum in vitro*[92]. WR 268,954 reduced the EC_{50} of chloroquine 90-fold in comparison to the chloroquine control. In chloroquine-sensitive isolates the effect was only additive.

There are numerous other promising leads, but their exploration requires time. The reality is dominated by the threat of losing the efficacy of affordable standard antimalarials in areas where a general change to the current third-line drugs is economically not feasible. Therefore all antimalarials, also the cheapest, should be used in the most effective and rational way in order to preserve their efficacy for the longest possible time. This may require major modifications in health care delivery but still be more acceptable than rising mortality from malaria.

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TREATMENT OF MALARIA

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Introduction

Malaria is the most important parasitic infection of man. Although eradicated from temperate zones, increasing numbers of travellers return each year from tropical countries, where the disease remains a major cause of morbidity and mortality. In these areas malaria is currently thought to affect approximately 250,000,000 people. Antimalarial drugs and antipyretics are the most commonly used drugs in the tropics. The drugs are consumed on a vast scale, and much of this use is unregulated. In many parts of Africa the majority of the population has chloroquine in the blood at any time. The treatment of malaria has changed over the past two decades as a result of declining drug sensitivity in *Plasmodium falciparum*, and a resurgence of the disease in tropical areas. Chloroquine resistance is now widespread in three continents, and resistance to sulphadoxine-pyrimethamine has developed rapidly in many countries. Mefloquine resistance has become a problem in S. E. Asia. This review concentrates on practical aspects of malaria treatment.

Diagnosis

The treatment of malaria should be based on a parasitological diagnosis where possible. Of course this is often not possible and treatment is given on suspicion, in febrile illnesses. When microscopic diagnosis is not available many non-malarial fevers are ascribed to malaria, severe malaria is often diagnosed incorrectly, drug resistance is usually underestimated, and the drug pressure to resistance is greater because of the widespread incorrect use of antimalarial drugs. The net cost to the community may be greater than the provision of a diagnostic service.

Malaria usually presents with non-specific and irregular fever, chills, headache and malaise. Abdominal discomfort and vomiting occur in approximately 20% and mild diarrhoea in 10% of cases. As the disease progresses the spleen enlarges and the patient becomes anaemic. Malaria is so common that any patient who has been in a malarious area within the previous two months (usual incubation period two weeks) should be considered to have malaria until proved otherwise. The diagnosis is confirmed by microscopy under oil immersion of stained thick and thin blood films . The parasites should be speciated (if in doubt consider as *P. falciparum*) and counted. In severe malaria the stage of parasite development, and the percentage of neutrophils containing malaria pigment should be noted also. The prognosis worsens with increasing parasite counts, more mature parasites, and more neutrophils containing pigment[1] (Table 1). A negative blood smear makes the diagnosis very unlikely, but if there is still diagnostic uncertainty the smear

Deep coma
Repeated convulsions
Respiratory distress (rapid, deep, laboured, stertorous, breathing often with intercostal recession)
Significant bleeding
Renal impairment (1) (serum creatinine > 250 μmol/L)
Hyperlactatemia (venous lactate > 5 mmol/μL)
Hypoglycemia (blood glucose < 2.2 mmol/L)
Plasma bicarbonate < 15 mmol/L
Elevated transaminases (> 3 times normal)
Parasitemia >500,000/μL *or*
>10,000 mature trophozoites (2) + schizonts/μL
≥ 5% of neutrophils contain malaria pigment

(1) The combination of deep jaundice and renal failure is particularly grave.
(2) Mature parasites in which pigment is visible under light microscopy.

Table 1. Severe malaria: Poor prognostic features.

should be repeated after 24 hours. Some patients will have received antimalarial treatment before admission and some of these may have cleared the peripheral parasitaemia, but pigment is still usually present in peripheral blood phagocytes if the patient is severely ill. A simple stick test based on a monoclonal antibody to *Plasmodium falciparum* histidine rich protein 2 has a diagnostic sensitivity similar to that of microscopy.

The management of uncomplicated malaria

The treatment of malaria depends on the severity of the infection, the age and degree of background immunity (if any), the likely pattern of antimalarial susceptibility, and the cost and availability of antimalarial drugs. For these reasons recommendations vary geographically, and should be reviewed constantly.

The three "benign" malarias *P. vivax, P. malariae* and *P. ovale* should all be treated with chloroquine. Although there is significant chloroquine resistance in *P. vivax* on the islands of New Guinea (Papua New Guinea, Irian Jaya)[2, 3] Sumatra and possibly some parts of the Solomon Islands, elsewhere it remains sensitive and responds rapidly. Other reports of chloroquine resistance are either unconvincing, or have confused recrudescence of the infection with relapse. *Chloroquine* is generally well tolerated, although it commonly produces pruritus in dark skinned patients, and may cause nausea, dysphoria and, very rarely, a transient neuropsychiatric syndrome or cerebellar dysfunction. Children often object to the taste of chloroquine, particularly in tablet form. Recent studies have shown that the traditional 3 day 25 mg (10, 10, 5) base/kg regimen can be compressed into 36 hours for convenience[4] (Table 2).

"Radical cure" with *Primaquine* 0.25 mg base/kg/ (0.33-0.5 mg/kg in Oceania and S.E. Asia) daily for 14 days is also required in *P. vivax* or *P. ovale* infections to eradicate persisting liver forms (hypnozoites) and prevent relapse. Primaquine may cause nausea and abdominal pain, particularly if taken on an empty stomach and, more importantly, oxidant hemolysis with methemoglobinaemia, anaemia, and sometimes haemoglobinuria. Patients with glucose 6-phosphate dehydrogenase (G6PD) deficiency are particularly vulnerable to oxidant haemolysis, and primaquine is contraindicated in severe variants of G6PD deficiency. Where mild variants of

	Uncomplicated malaria (Oral)	Severe malaria (*) (Parenteral)
Chloroquine	10 mg *base*/kg followed either by 10 mg/kg at 24 hrs and 5mg/kg at 48 hrs *or* 5 mg/kg at 6, 12 and 18 hrs. Total dose 25 mg base/kg. For *P. vivax* or *P. ovale* add primaquine 0.25 mg base/kg daily for 14 days (**) for radical cure.	10 mg *base*/kg by constant rate infusion over 8 hrs followed by 15 mg/kg over 24 hrs *or* 3.5mg base/kg by intramuscular or subcutaneous injection every 6 hrs. Total dose 25 mg base/kg.
Sulfadoxine - pyrimethamine	20/1 mg/kg single oral dose (3 tablets for an adult).	
Mefloquine	15 mg *base*/kg. In mefloquine resistant areas or for non-immunes give second 10 mg/kg dose 8-24 hrs later. For semi-immunes 15 mg *base*/kg single dose.	
Quinine	10 mg *salt*/kg 8 hourly for 7 days combined with tetracycline (†) (4 mg/kg) four times daily *or* doxycycline 3 mg/kg once daily for 7 days.	20 mg *salt*/kg by intravenous infusion over 4 hrs (‡) followed by 10 mg/kg infused over 2-8 hrs every 8 hrs.
Quinidine		10 mg *base*/kg infused at constant rate over 1-2 hrs followed by 0.02 mg/kg/min, with close electrocardiographic monitoring.
Artesunate	In combination with 25 mg/kg mefloquine as a split dose, 10-12 mg/kg is given in divided doses over 3-5 days (e.g.4 mg/kg for 3 days or 4 mg/kg followed by 1.5 mg/kg/day for 4 days). If used alone, the same dose is divided over 7 days (usually 4 mg/kg initially followed by 2 mg/kg on days 2 and 3 followed by 1 mg/kg on days 4-7).	2.4 mg/kg i.v. or i.m. stat followed by 1.2 mg/kg at 12 and 24 hrs and then daily.
Artemether	Same dose regimen as artesunate.	3.2 mg/kg i.m. stat followed by 1.6 mg/kg daily.

N.B.: In severe malaria quinine or quinidine should be used if there is any doubt about the chloroquine-sensitivity of the infection.

(*) Oral treatment should be substituted as soon as the patient can take tablets by mouth.
(**) In Oceania and S. E. Asia the dose should be 0.33-0.5 mg base/kg.
(†) Tetracycline or doxycycline should not be given to pregnant women or children < 8 years old.
(‡) Alternatively 7 mg salt/kg can be infused over 30 minutes followed by 10 mg salt/kg over 4 hours[5].

Table 2. Antimalarial Drugs: Recommended doses for treatment.

G6PD deficiency are common, primaquine (0.8 mg base/kg: adult dose 45 mg) should be given once weekly for six weeks for radical cure[6]. In endemic areas where reinfection is common, radical cure with primaquine is not indicated. Chloroquine resistant *P. vivax* responds to mefloquine and primaquine (which also possesses asexual stage activity). The choice of treatment for *P. falciparum* depends on the antimalarial sensitivity in the area where the infection was acquired. Known sensitive infections (e.g. from North Africa, Central America or the Middle East) should be treated with *chloroquine*. Where there is low-grade chloroquine resistance, *amodiaquine* (Total dose 35 mg base/kg/day over 3 days) is a more effective alternative. Amodiaquine is well tolerated. Children prefer the taste to chloroquine. The risks of life-threatening adverse effects (agranulocytosis, hepatitis) which occured in approximately 1:2000 people who took amodiaquine for prophylaxis, are considered much lower in treatment, although firm data are lacking.

Chloroquine-resistant infections from most of Africa and some parts of Asia and S. America usually respond to the combination of a long acting *sulphonamide* (usually sulphadoxine) and *pyrimethamine*. This is a well tolerated single dose treatment. Serious sulphonamide toxicity (severe skin reactions, cytopenias, haemolytic anaemia, neuropathy, hepatitis) which was estimated to have occured in 1:7000 people who took the combination for prophylaxis is considered rare in treatment, although again firm data are lacking. In practice chloroquine is still extensively used even in areas with established resistance, because the alternatives are too expensive. At the time of writing only Malawi and Kenya of the African countries have changed their National Policy to first line treatment with sulphadoxine-pyrimethamine, although chloroquine resistance has spread from the east to the west coast of the continent. The therapeutic response to chloroquine, or any other antimalarial drug, is always better in semi-immune subjects compared to non-immunes so chlorquine is still useful in these resistant areas, but the incidence of severe anaemia in infants and young children inevitably increases as drug susceptibility declines. Thus the full impact of chloroquine resistance in areas of high transmission may not be fully appreciated unless there are longitudinal data on the incidence of severe anaemia in young children. Unfortunately resistance to sulphadoxine-pyrimethamine has developed rapidly in some areas (particularly S. America and S. E. Asia). For multi-drug resistant strains of *P. falciparum* the choice of treatment lies between mefloquine, halofantrine, or quinine plus tetracycline (clindamycin is used in some countries).

Mefloquine is cleared slowly ($t_{½ß}$ 2-3 weeks) and either a single or split dose is sufficient for cure[7]. An initial 15 mg base/kg dose should be followed 8-24 hours later by 10 mg/kg. Most data on doseage refer to the formulation Lariam^{R} (Roche). As oral bioavailabilty varies considerably with mefloquine, more information is required on other formulations. Mefloquine treatment is relatively well tolerated although nausea, vomiting, giddiness, weakness, dysphoria, feelings of dissociation, mental clouding, and nightmares are all common. Rarely (1:1700 with 15 mg/kg, 1:1200 with 25mg/kg) more serious self-limiting neuropsychiatric reactions may occur[8, 9]. The incidence of serious neuropsychiatric reactions is higher in European and African patients compared with Asian patients. Although early (< 1 hour) vomiting is more common in children, the other adverse effects of mefloquine are more common in adults[10, 11], and interestingly are complained of more frequently by women than men.

Halofantrine is more active and better tolerated than mefloquine, but it has poor and variable bioavailability (improved by fats). Halofantrine induces a significant concentration-dependent delay in atrioventricular conduction and ventricular repolarisation[12], that has cast a shadow over its future role. Halofantrine should not be given to patients with a long QT_c interval or those taking drugs likely to delay repolarisation. Neither halofantrine nor mefloquine should be used to treat early recrudescences of malaria (< 28 days) following high dose mefloquine as their re-

spective cardiac and central nervous system effects are increased.

Oral treatment with the Cinchona alkaloids *quinine* or *quinidine* is not well tolerated. These venerable compounds are extremely bitter and reliably induce the symptom complex of cinchonism (nausea, dysphoria, tinnitus and high tone deafness). Fortunately more serious toxicity is rare. Although the quinine plus tetracycline or doxycycline combination remains >85% effective nearly everywhere[13], compliance with the five to seven day courses of treatment required in resistant areas is poor. In areas where the *P. falciparum* parasite is more sensitive then a three day course of quinine can be combined with seven days of tetracycline. Unfortunately in most of these areas the brunt of malaria is in children who cannot take tetracyclines. Short courses of quinine (< 5 days) are not effective, and even if compliance is good, courses of 5 days treatment have significant failure rates.

The derivatives of *artemisinin* (qinghaosu) obtained from qinghao or sweet wormwood *(Artemesia annua*) and developed in China, are the most rapidly acting of all antimalarial drugs. In both severe and uncomplicated malaria they have given consistently faster fever and parasite clearance than other antimalarials without evident toxicity[14, 15]. Three compounds have been used; the parent artemisinin, and two more active derivatives, a water soluble hemisuccinate, artesunate, and an oil soluble ether, artemether, both of which are metabolised to a common biologically active metabolite dihydroartemisinin (DHA). Indeed artesunate can be considered a pro-drug for DHA. In some parts of S. E. Asia (particularly the Eastern and Western borders of Thailand) treatment failure rates with high dose mefloquine alone in falciparum malaria now exceed 40%. In these circumstances oral artesunate given for 3-5 days in combination with mefloquine (25 mg/kg) still remains highly effective with cure rates still in excess of 85%[16, 17]. If the artemisinin derivatives are used alone (e.g. for the treatment of recrudescence following mefloquine) then they should be given for seven days. The main concerns surrounding these extraordinary drugs are the unresolved issue over neurotoxicity, and the difficulty in regulating drugs which are relatively cheap, very well tolerated, and highly effective. Already these compounds are available in the market place. This is a prescription for abuse, and the development of resistance. The development of resistance to the artemisinin compounds would be a disaster, as these drugs are still effective against multi-drug resistant falciparum malaria, and also offer the potential to reduce the mortality of severe malaria. In animal models these drugs, particularly the oil-soluble ethers artemether and arteether, have produced an unusual, selective pattern of damage to brain stem nuclei. There is no evidence of this to date in man despite extensive use of the drugs, but studies are ongoing and the possibility remains that they may be neurotoxic.

Children and pregnant women

Children tolerate antimalarial drugs relatively well. Mefloquine is not recommended for children < 15 kg, but this is a policy of caution because of limited published experience. Our own experience suggests there are no additional risks, although infants are more likely to vomit the drug. In resistant areas it has been recommended that quinine should be given in an eight day course to young children, increasing the dose in the last four days to 15 mg/kg three times daily. Primaquine should not be given to newborns or pregnant women. Chloroquine, sulphadoxine-pyrimethamine, quinine and quinidine are considered safe in therapeutic doses in all trimesters of pregnancy, although there is a theoretical risk of kernicterus if sulphadoxine is used in the third trimester. There is increasing evidence that mefloquine is safe in the second and third trimesters. Although there is very little information on the use of the artemisinin derivatives in pregnancy, the general consensus is that they too should be used if they are indicated (i.e. for mefloquine resistant falciparum malaria), except in early pregnancy where alternative treatment (quinine) is still preferred. Apart from primaquine and halofantrine (where there are no data), the other anti-

malarials can all be used in women who are breast-feeding.

Practical considerations

There are no pediatric formulations of mefloquine, the artemisinin derivatives, primaquine or, in many countries, quinine or chloroquine. When treating children, particular care should be taken to ensure the correct doses are given and retained. Early vomiting is common, particularly following the administration of mefloquine or quinine to infants, and is more likely with high fever. Patients should be cooled (acetaminophen, fanning and tepid sponging) before receiving oral antimalarial treatment and then observed for one hour. If vomiting occurs within one hour the full dose should be repeated (for mefloquine we give half the dose for vomiting between 30 and 60 minutes). If vomiting occurs after one hour, it is not necessary to re-administer the drugs. Postural hypotension is common in uncomplicated malaria, and is exacerbated by the quinoline antimalarials. Febrile patients should be nursed horizontally, and should take great care if they get up rapidly from their beds. Mothers should be discouraged from carrying febrile babies vertically immediately after parenteral quinine or chloroquine has been given.

Severe *Falciparum malaria*

Management

Whereas *P. vivax*, *P. ovale* or *P. malariae* are very rarely fatal, *P. falciparum* infections may progress rapidly to a lethal multisystem disease. The clinical manifestations of severe malaria depend on age[18]. Hypoglycemia, commonly accompanied by lactic acidosis, convulsions, and severe anaemia are relatively more common in children[19], whereas acute renal failure, jaundice and pulmonary oedema are more common in adults. Cerebral malaria (coma), shock and respiratory distress leading to respiratory arrest occur at all ages. The state of hydration on admission is very variable. The physician must tread a thin dividing line between over and under-hydration. Adults in particular may develop non-cardiogenic pulmonary edema and are vulnerable to fluid overload, yet dehydration and hypovolaemia contribute to hypotension and shock, and may hasten the development of acute renal failure from acute tubular necrosis[20]. After rehydration the central venous pressure should be maintained at approximately 5 cm of water (pulmonary artery occlusion pressure < 15 mm Hg). When hypercatabolic acute renal failure develops with other evidence of vital organ dysfunction, dialysis or haemofiltration should be started early. Renal function returns with a median time to restoration of urine flow (> 20 ml/kg/day) of 4 days, although some patients will require dialysis for more than a week. Hypoglycemia occurs in approximately 8% of adults and 25% of children[19, 21]. After rehydration a maintenance 5-10% glucose infusion should be given to all patients but the blood glucose should still be checked frequently. Unconscious patients (cerebral malaria) should be nursed on their side, and a lumbar puncture performed to exclude bacterial meningitis. There has been muchos debate over the role of raised intracranial pressure, particularly in childhood cerebral malaria. The general consensus is that paroxysmal rises in intracranial pressure may occur but that these rarely result in lethal brain stem displacement. Empirical treatment with osmotic agents is *not* recommended. Convulsions should be treated promptly with intravenous benzodiazepines. Use of prophylactic intramuscular phenobarbital reduces the incidence of seizures, but the optimum dose remains to be determined. Haemolysis is extensive and anaemia develops rapidly. Blood transfusion should be given if the haematocrit falls below 20%. Fresh blood transfusion is preferable, particularly if there is significant bleeding secondary to disseminated intravascular coagulation (≈ 5% of patients with severe malaria) or stress ulceration. Bacterial infections are common; pneumonia is particularly likely if the duration of coma exceeds three days, urinary tract infections complicate indwelling catheter drainage, and spontaneous (usually gram negative) septicaemias may occur occasionally. In Africa non-typhoidal systemic *Salmonella* infections may develop in otherwise uncomplicated malaria[22]. If a pa-

tient with severe malaria deteriorates suddenly without an evident cause, hypoglycaemia should be excluded, blood cultures drawn, and empirical broad spectrum antimicrobial treatment started. Vital signs including coma score, urine output, blood glucose and, if possible, lactate, and arterial pH and gases should be monitored as frequently as possible. After starting antimalarial treatment, the parasite count should be measured at least twice daily in all patients. If the parasite count has not fallen by 75% at 48 hours, it should be re-checked, and if confirmed, then the antimalarial should be changed.

Antimalarial Treatment

Chloroquine

In those few remaining places where there is not significant resistance, then parenteral chloroquine should be given as it is intrinsically superior to quinine[23]. If there is any doubt, then quinine or quinidine should be used. Chloroquine should be given by controlled-rate intravenous infusion (*never* injection). Intramuscular or subcutaneous administration is a satisfactory alternative as absorption is rapid, bioavailability exceeds 80% even in severe malaria, and the injections are not painful[24]. Provided it does not enter the circulation too rapidly, either because the rate of intravenous administration is too fast, or the individual intramuscular or subcutaneous injection dose is too large (> 3.5 mg base/kg), then hypotension will not occur, and parenteral chloroquine is very well tolerated.

In some countries amopyroquine (a compound similar to amodiaquine) is available and administered by intramuscular injection. Although a parenteral formulation of sulphadoxine-pyrimethamine exists it should not be used in the sole management of severe malaria.

Quinine and Quinidine

The alkaloids derived from the bark of the Cinchona tree constitute the mainstay of the antimalarial pharmacopoeia, as they have for over three hundred years. Quinidine, the dextrorotatory diastereomer, is more active than quinine, but it is also more cardiotoxic and more expensive. In the United States parenteral quinidine is recommended for severe malaria, as it is available widely (as an antiarrhythmic)[25]. Elsewhere quinine is used. The Cinchona alkaloids are effective against all species of malaria including chloroquine-resistant strains of *P. falciparum*[18]. Both drugs have narrow therapeutic ratios, although serious cardiovascular or nervous system toxicity during antimalarial treatment is most unusual. Quinine and quinidine should be given by intravenous infusion, *never* by bolus injection, otherwise fatal hypotension may ensue. The principal adverse effect in severe malaria is hyperinsulinaemic hypoglycaemia[26, 27]. Iatrogenic hypoglycaemia usually develops after at least 24 hours of treatment, and is a particular problem in pregnant women[28]. Quinidine has similar effects on insulin secretion, but a four-fold greater effect on the heart[29]. Electrocardiographic monitoring is required so that infusion rates can be stopped if the rate corrected QT interval is prolonged by > 25% of the baseline value or exceeds 0.65. Quinidine is a difficult drug to use as cardiovascular effects are common. Routine EKG monitoring is not necessary when quinine is used.

The pharmacokinetic properties of the Cinchona alkaloids are altered considerably in malaria, with a contraction in Vd and a reduction in systemic clearance that is proportional to disease severity[30]. In severe malaria doses should be reduced by 30-50% after the third day of treatment to avoid accumulation in patients who remain seriously ill. The therapeutic range for *free* drug depends on the sensitivity of the infecting malaria parasites. This has not been defined precisely, but is probably in the range of 1-2 mg/L (South East Asian parasites tend to be more resistant), which corresponds to total plasma concentrations of approximately 4-8 mg/L for quinidine, and 8-20 mg/L for quinine. Binding, principally to the acute phase protein α_1-acid glycoprotein is increased in malaria (from approximately 80% to 90% for quinine)[31]. This probably explains why total plasma concentrations of quinine (> 10 mg/L) that have been associated with blindness and deafness following

self-poisoning, are usual in the treatment of severe malaria and do not cause these adverse effects. It has been suggested that the initial loading dose o quinine could be reduced in areas with drug sensitive parasites, but as there is very little evidence of serious toxicity even in patients with inadvertent pretreatment, and reduction in dose would risk potentially dangerous undertreatment. Dose reduction is not recommended.

Where intravenous infusions cannot be given, quinine dihydrochloride, diluted to of 60-100 mg/ml, should be administered by deep intramuscular injection to the anterior thigh. The initial loading dose should be split and given to both legs. Intramuscular bioavailability is good (≈ 90%) even in severe malaria[32, 33]. Quinine injections are painful and, if undiluted, occasionally produce sterile abcesses.

Artemisinin (Qinghaosu)

Artesunate is unstable in solution and is therefore dispensed as a dry powder of artesunic acid together with an ampoule of 5% sodium bicarbonate. The two are mixed and the solution is given by intravenous or intramuscular injection. Artemether is more stable. It is formulated in groundnut oil, and is given by intramuscular injection. In recent studies[34] artemisinin rectal suppositories have proved as effective as the parenteral drugs. Thus effective antimalarial treatment can be given for severe malaria in rural settings where injections cannot. Artemisinin and its derivatives appear to have a broader "time-window" of effect than other antimalarials during the 48 hour asexual life cycle. Antiparasitic effects on the younger "ring-form" parasites lead to their clearance and prevent development to the more mature pathogenic forms that induce the parasitised erythrocytes to adhere to uninfected cells (rosetting) or to vascular endothelium (cytoadherence). Artesunate is the most rapidly acting of the available compounds, possibly because it is immediately bioavailable as DHA following intravenous injection, and is absorbed rapidly following oral or intramuscular administration. Over a million patients have been treated with the artemisinin derivatives. No significant toxicity has been reported[14]. However, in experimental animals, artemether, arteether, and the metabolite DHA have induced an unusual and selective pattern of damage to some of the brain stem nuclei[35]. The relevance of these findings to their use in man is unresolved, but remains an area of concern.

Ancillary Treatments

Many ancillary treatments have been suggested and tried in severe malaria, but only antipyretics (acetaminophen) and anticonvulsants (prophylactic phenobarbital), and exchange transfusion have been supported by sufficient evidence to warrant their use[25, 5, 36]. The role of anti-TNF antibody, desferrioxamine, mannitol, prostacyclin, dextran, pentoxyifylline, and N-acetylcysteine is uncertain, aspirin and hyperimmune serum have been shown to confer no benefit, and heparin, cyclosporine, and high dose corticosteroids are probably harmful. Exchange transfusion should be performed if there are adequate facilities, and the parasitemia exceeds 15%, and should still be considered with parasitemias in the 5-15% range, particularly if there are other signs of poor prognosis (Table 1).

The future[37]

Nature provided us with the Cinchona alkaloids and qinghaosu, the Second World War prompted research that led to the introduction of chloroquine, proguanil (chloroguanide), and led eventually to amodiaquine and pyrimethamine. The conflict in Viet Nam brought mefloquine and halofantrine. These drugs are all we have available now to treat malaria. It is difficult to see where the next generation of antimalarial drugs will come from. Even though malaria now affects about 250 million people and kills between one and three million children each year, there is little pharmaceutical industry interest in developing new antimalarial drugs as the risks are significant, but the returns on investment are so low; much of the world's malaria occurs in countries with an annual per-capita expenditure on health of less than $10. However, if drug resistance in *P. falciparum* continues to develop at the current rate, then it is possible that we may be confronting untreatable malaria in parts of South East Asia by the beginning of the next millenium[37].

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MALARIA CHEMOPROPHYLAXIS

M. Corachan

Introduction

Protection against malaria infection has two major aspects: personal protection against mosquito bites (repellents, bednets, personal outfit, etc) and the use of chemoprophylactic drugs. The former is being increasingly recommended in traveller's advice and used in control strategies against the infection; the latter is frequently and passionately debated.While in endemic areas only selected groups in specific situations will use chemoprophylaxis, any tourist visiting these areas should use one or a combination of prophylactic drugs together with the above mentioned measures against mosquito bites.

This is a controversial field: what drugs for which areas, who should take them and for how long, what area the indications and contraindications, the incidence of serious side effects; these questions are frequently discussed. In general and whatever the strategy, it is advisable for all travellers to malaria infested areas to protect themselves but the same cannot be said for the resident population in endemic areas where clear selection of population groups is mandatory for major drawbacks can be expected from a non-selective attitude. In these countries the choice of a particular drug will be limited, because their national policies will decide which drug is used for chemoprophylaxis.Statistical data from the World Tourism Organization at the beginning of this decade showed that nearly 30 million people from non malaric countries visit malaria endemic areas yearly[1]. The matter is therefore of major importance in terms of international health. Although the caucasian tourist seems to be the main target group for chemoprophylaxis, other ethnic groups with years of residence in Europe or the US, who go and visit relatives in malarious areas increasingly appear in statistics of imported malaria in the developed world[2]. In many countries a high percentage of travellers seek health advice from their travel agencies[3, 4], which usually tends to minimize the risks. Surveys carried out at different european airports show that travellers leave their countries with poor information (if any) about malaria prophylaxis. A major effort is needed for updating the training of general practitioners and travel clinics personnel. For the new generations, international health should find its place in medical schools and spark an awareness of these problems in future young doctors and other health personnel.

Protection against mosquito bites

From a practical point of view, a tourist visiting endemic areas for short periods should protect himself with mosquito repellents and bednets, while the local pop-

ulation should ideally be protected with impregnated bednets.

Mosquito Repellents

Synthetic pyrethroid coils give out an effective smoke during an 8 hs. period, but concern is growing about the potential lung damage inflicted after prolonged exposures. The spraying of walls and ceilings, for years a major asset of the anti malaria national campaigns, is less used nowadays, for purely economic reasons because residual insecticides such as DDT have still a place in fighting mosquito in the home rooms and are harmless to humans[5]. DDT application in agriculture is more controversial.

Bednets

Bednets impregnated with the pyrethroid insecticides can last for about six months[6]. Their effect on morbidity and mortality of children was well proved through a controlled trial in the Gambia[7] and has been confirmed since then by many authors in different geographic and social settings. Considered by many as the best and more reliable tool for controlling malaria at present. Keeping the bednets effective requires minimum care activities: if not repaired they end up being real mosquito traps, if washed they will loose part of its repellent potential. Therefore the active involvement of individuals and of the community as a whole is required for any bednets programme to be successful.

Repellents

Vapour released from commercial tablets or coils are effective when used in enclosed places but of doubtful effect when used at the open air. In Kenya, local herbs used by villagers have been found to be as effective as the commercial coils[8].

Di-ethyl toluamide is the most used type of chemical repellent. It is more effective in its alcohol solution with concentrations ranging between 25 and 50%. It has to be applied in the areas where most mosquitoes bite: low third of inferior extremities and the uncovered body areas.

Chemoprophylaxis for the local population in endemic areas

The use of chemoprophylaxis for residents in endemic areas is justified in three obvious at risk situations (infants, pregnancy and especially primigravidae, and the returning migrants) where the risks of not giving malaria protection will be too high.

1 - In infants and young children in whom malaria is potentially lethal, directly (cerebral malaria) or indirectly (severe anaemia). No immunity or partial immunity is directly related to the clinical severity present in those cases. It has been rightly pointed out[9] that more work is needed to evaluate whether the beneficial effects of chemoprophylaxis on the infant, are outweighted by a reduction of transplacental transfer of IgG. On the other hand the potential rebound effect after stopping chemoprophylaxis in infants has been shown to be true in terms of increase in the number of clinical attacks but there was no detectable effect on mortality[10].

Despite the difficulties of sustainability for achieving a wide coverage of those at risk groups, the many arguments in favour of chemoprophylaxis outweight those against.

As during the malaria attack, iron reduced absortion and sequestration occur the infection contributes to anaemia in this age group. It has been shown that combining both chemoprophylaxis and insecticide treated bednets reduces the malaria infection as well as the number of anaemia cases. This approach is most advisable when feasibility is present, as it could reduce considerably the number of blood transfusions given at that age group in many tropical countries.

2 - Pregnant women and especially primigravidae from endemic areas have a higher incidence of anaemia and clinically severe malaria, but this incidence is reduced in women taking antimalarials[11].

However, specific IgG levels in pregnant women are not different in those with or without chemoprophylaxis. Targeted chemoprophylaxis is now recommended in pregnancy situations such as primigravidae and anaemic women[12] as well as the grand multigravidae with more than five pregnan-

cies[13]. In endemic areas the use of chemoprophylaxis during pregnancy will determine the outcome. In this way there will be a significant reduction of low weight children, abortions and placentia previa. In the case of primigravidae, infant mortality can be reduced by a 50%[13].

3 - Migrants to zones of high endemicity coming from countries with no malaria (foreign residents) or from areas of the same country with a low prevalence of malaria. In both cases the situation of practically no immunity, parallels these groups with the caucasian travellers as to the risk of acquiring malaria.

General considerations

Provided the malariometric data of the area are well defined, the pros and cons of the strategy have been weighted and a reasonable network of health workers is available for the distribution of this prophylaxis, mortality and morbidity of this infection can undoubtedly be considerably reduced. A strategy of wide distribution is likely to produce major drawbacks[14].

Sustainability in terms of economy and drug delivery is a serious obstacle and if restrictions are to be implemented group 3 (migrants) will receive a lower priority than infants and primigravidae. In those groups, the safer and cheapest drugs are used at MCH clinics, i.e. Chloroquine or Paludrine or both, in order to facilitate massive distribution to those groups. By restricting chemoprophylaxis to those selected groups, the drug pressure over the parasite is reduced and resistance is less likely to form[15, 16]

Dosage of these and other drugs are presented in the following tables:

Drug	Dosage adults
Chloroquine (Cl)	300 mg/week (5 mg/kg.)
Proguanil (P)	200 mg/day
Cl. + P.	300 mg/wk + 200 mg/day
Dapsone (DDS) + Pyrimethamine	(100 mg + 12.5 mg) per week
Doxycicline (*)	100 mg/day
Mefloquine (*)	250 mg/week

(*) Not to be used in pregnancy or in children less than 8 years of age.

Table 1. Main chemoprophylactic drugs used in Malaria prevention.

Drug	Dose	Observations
Chloroquine	5 mg/kg of the base product	Syrup available in some countries
Proguanil tabs: 100 mg.	50 mg/day: 6/14kg 75 mg/day: 15/29 kg 100 mg/day: 30/40kg	To be taken together + a weekly Chloroquine in indicated areas
Mefloquine tabs: 250 mg (*)	15/19 kg: 1/4 Tab. 20/30 kg: 1/2 Tab. 31/45 kg: 3/4 Tab.	Single weekly dose only if weight > 15 kg
Doxicicline tabs:100 mg (*)	2 mg/kg /day	Only if > 8 ys. in multiresistant zones
Pyrimethamine + Dapsone (**)	0.2 mg/kg + 1.5 mg/kg	A syrup formulation exists (Wellcome)

(*) Used mainly by tourists.
(**) Not recommended if < 6 weeks.

Table 2. Chemoprophylaxis for children. Available drugs.

Traveller's chemoprophylaxis

General comments

For the purpose of malaria chemoprophylaxis, a short term visitor to an endemic area will always be considered as a traveller whether he has always been non-immune or is an immigrant who has lost his acquired immunity after living for several years in a non endemic area. The best advice will be to recommend a safe and efficient drug adapted to the geographic area of travel, with no clear contraindications for the particular traveller, and finally the importance of compliance should be stressed without which the efficacy of the drug is considerably reduced. The importance of starting before reaching the transmission zone and of the 4 weeks drug intake after returning to Europe cannot be overemphasized. In this way we are able to prevent *P. falciparum* infections during the last days before departure. Any experienced traveller to Africa will be aware of the fact that many return flights from this continent are night flights where tourists spend a considerable amount of time in airports generally offering little protection against mosquitos.

This said, travellers should also be aware that there is no wonder pill giving absolute protection, simply because there is no drug that can interfere with the development of sporozoites just after they have been inoculated by the *Anopheles* mosquito.Minor adverse reactions to chemoprophylactic drugs against malaria have been recorded by several groups of researchers, with similar rates for whatever drug or combination of drugs used[17, 18].

Our study on spanish travellers confirmed the above data as well as those of compliance (similar to the Chloroquine + Paludrine group). However we realized that non compliance in the Mefloquine group was due to the travellers' feeling of uneasiness when having clinical symptoms in relation with the CNS: anxiety, hyperkinesia and poor mental concentration, all experienced by patients under Mefloquine and not recorded in travellers taking the other drugs[19].

Geographical areas of drug utilization

The following countries have been listed after consulting sources corresponding to different groups of epidemiological surveillance based in Europe: Conseil Supérieur d'Hygiène Publique de France[20], the Public Health Laboratory Service (LSHTM)[21] and the WHO official publications[22]. The distribution of the species can be consulted in other chapters.

Countries where Chloroquine remains the first choice

AFRICA - Algeria, Cape Verde, Egypt (el Fayoum), Lybia, Mauritius and Morocco.

AMERICA - Argentina (North), Belize, Bolivia (South), Costa Rica, Guatemala, Haiti, Honduras, Mexico, Nicaragua, Paraguay (East), Perú (except in the areas bordering the neighbour countries), Dominican Republic (area bordering with Haiti), El Salvador and Panama (North).

ASIA - Azerbaïdjan (South), Tadjikistan (South), China (North-East).

MIDDLE EAST - Iran (except South East), Iraq (Northern areas below 1500 mt. and Basrah prov.) Syria (limited foci in the North), Turkey (Çukurova, Amikova and Anatolia).

Countries where Cl+P or Mefloquine are used as first choice

AFRICA - Benin, Botswana, Burkina Faso, Gambia, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Liberia, Madagascar, Mali, Mauritania, Namibia, Niger, Republic of South Africa (Transvaal, Natal) Senegal, Sierra Leone, Somalia, Tchad and Togo.

ASIA - Afghanistan, Bhutan, India, Indonesia (except Irian Jaya) Malaysia (hinterland foci), Nepal, Pakistan, Arab Emirates, and Yemen.

Countries where Mefloquine will be the first choice (*)

AFRICA - Angola, Burundi, Cameroon, Central Africa Republic, Comores, Congo, Djibouti, Eritrea, Ethiopia, Equatorial Guinea, Gabon, Kenya, Malawi, Mayotte, Mozambique, Nigeria, Rwanda, Sao Tomé & Principe, Sudan, Swaziland, Tanzania, Uganda, Zaire, Zambia and Zimbabwe.

AMERICA - Bolivia (North), Brazil (risk areas: Amazonia, Mato Grosso, Rondonia, Parà, Maranhao), Colombia, Ecuador,

French Guyana, Guyana, Panama (South), Perú (East), Suriname and Venezuela.

ASIA - Bangladesh, Cambodia, China (Yunnan, Hainan) Laos, Myanmar Republic, Thailand (in forest areas but not in the Cambodia and Myanmar borders), Vietnam, Malaysia (Sabah). In any patients having contraindications for Chloroquine and/or Mefloquine, Doxicycline (100 mg/day) can be used. Doxicycline will be also the drug of choise for the bordering areas of Thailand with Cambodia and Myanmar Republic.

(*) Except for trips longer than 6 months.

The South Pacific Area

In this geographical area, Mefloquine as well as the combination Chloroquine-Pyrumethamine/DDS (Maloprim®) can be used.

The countries with endemic malaria are the following: Indonesia (Irian Jaya), Papua New Guinea, Vanuatu.

Further to the East there are to date no other countries in the Pacific where malaria is endemic.

Drugs. Their adverse reactions and contraindications

We will now review the drugs (or combination of drugs) individually:

Chloroquine

A 4-aminoquinoline which is a blood schizontocide of rapid action. Despite the widespread status of chloroquine resistance it is still widely used in tropical areas as the drug of choice in MCH clinics where chemoprophylaxis is implemented against the selected target groups.

Secondary effects on the retina only occurs with prolonged use. The development of neuroretinitis is a possibility that requires more than 50 grs accumulated over several years (1 year accumulation is usually >15 grs.). However a number of american missionaries have been evaluated after more than 20 ys of accumulated chloroquine and no one case of retinitis has been diagnosed[23].

The drug is also gametocytocidal against *P. vivax, P. ovale* and *P. malariae*.

At present the usefulness of this drug is restricted to areas of Central America and the Caribbean (Haiti,Dominican Republic) where the *P.falciparum* strains are sensitive to Chloroquine, as well as in the Middle East.

Adverse effects and contraindications

The drug is not recommended in patients with psoriasis or with an active gastroduodenal ulcer. In both cases, a relapse of the condition is possible. There have been occasional reports in patients with a low seizure threshold therefore the drug is not recommended in patients with a past personal or family history of epilepsy[24].

The most frequently reported adverse effects belong to the gastrointestinal tract: abdominal pain, vomiting, nausea. Corneal crystal deposition without affecting the vision is frequently seen in residents after few months of taking the drug.

Proguanil

A slow acting schizonticide used only as a prophylactic drug. As safe in pregnancy as chloroquine but a folic acid supplement must be added.

Adverse Effects and contraindications

This is the safest of all chemoprophylactic drugs, with practically no contraindications. Minor gastrointestinal problems like heartburn or epigastric pain have been reported mainly by people who take the drug while fasting. Mouth ulcers are definitely the more disturbing of the adverse effects reported.

Chloroquine + Proguanil

4-aminoquinoline and a biguanide or type 2 antifolate that can inhibit any stage of the malaria parasite. This combination is still a first choice in a number of West African countries according to our English and French sources as well as in areas of Mefloquine choice when this drug is to be administered for long periods.

Adverse effects and contraindications

The same as for both drugs taken individually. Mouth ulcers disappear after re-

ducing the usual dose from 200 mgs to 100 mgs without reducing the expected protection against the infection.

D.D.S. + Pyrimethamine

A sulphone which is a type 1 antifolate with a slow schizontocidal action is associated here with a type 2 antifolate. First recommended for malarial areas of the South Pacific and Indonesia it has now extended its geographical action to areas where both Chloroquine and Mefloquine resistance coexist. Having been found safe as prophylaxis in Gambian children, it is now used in other areas of Africa for the same purpose.

Adverse effects and contraindications

When the weekly dosage was doubled at two tablets per week, cases of potentially fatal agranulocytosis have been reported (REF). Persons with hereditary NADH methaemoglobin reductase deficit can develop clinical methaemoglobinemia.

People with known susceptibility to sulphonamides compounds cannot take the drug as Dapsone (D.D.S) could induce potentially fatal reactions of the dermato-exfoliative type. Sporadic severe cases could even be accepted depending on the severity of the malaria situation as a whole. The drug has been used extensively in infants and children of the subsaharan zone, and no serious side effects have been reported[25].

Mefloquine

An arylaminoalcohol (quinoline methanol) is a blood schizontocide for all species of malaria parasites. Despite his relatively recent emergence its indiscriminated utilization is causing a rapid dissemination of resistance to this drug. This is particularly true for the area of the Thailand/Cambodian frontier and it is feared that the same phenomenon will soon occur in other tropical areas. The sole advantage of this dissarray is that Chloroquine sensitivity is recovering in the areas where it has not been used for some time[26]. French authors have shown Mefloquine resistance in some areas of West Africa where the drug has not been used yet[27].

Adverse effects and contraindications

The drug is not indicated in patients with personal or family history of epilepsy, patients under hypnotic and antidepressant drugs as well as in persons with cardiac arrythmias.

The prevalence of adverse effects is similar as with other drugs according to several studies. This, together with the easiness of the regimen and its efficacy[28] were determinant for the widespread use of this drug. Compliance should be easier with Mefloquine that has to be taken only once a week, however the frequently reported CNS symptoms (insomnia, vertigo, hyperkinesia etc) often leads to the patient's discontinuation of the treatment. The risk for developing acute psychotic reactions under chemoprophylactic regimes has been evaluated at 1/12000 persons[29]. A case of Mefloquine induced grand mal seizure in a non epileptic patient has been recently described[30].

Cases of *P. falciparum* infections in Dutch short time visitors to the tropics under Mefloquine prophylaxis indicate that the drug should be started earlier than usually prescribed or a loading dose should be given instead, if adequate protecting circulating levels of the drug are to be achieved[31].

At present there is no consensus concerning the length of time in which the drug should be used. Not more than 12 months according to most European authors and several years according to US publications.

Doxycicline

Antibiotic with slow marked actions at the blood stage of the parasites. Recommended in areas of multiple drug resistance such as the Thailand/Cambodia or the Thailand/Myanmar borders especially for short term travellers. The drug can also be recommended in patients with clear contraindications for Mefloquine use when that was the drug of choice. As with other tetracyclines, doxycicline should not be used in children nor in pregnant or lactating women.

Adverse effects and contraindications

Some have been already mentioned.

Vaginal candidiasis, oesophageal ulcers and intestinal flora modifications are some of the problems inherent to the administration of this drug together with the well known photosensitivity reactions. To what extent its prolonged administration could interfere with oral contraceptive medication is still unknown.

Finally it should be remembered that nowadays none of the regimens described above is one hundred per cent effective in supressing a *Plasmodium* infection; however their important role in reducing morbidity and mortality when used both as a control tool or in travel medicine cannot be denied. Another important point is the demonstration that for each drug regimen, compliance with the prophylaxis strongly influenced the degree of protection achieved[32].

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MALARIA CONTROL STRATEGIES IN AFRICA

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Introduction

Strategy is the art of planning and managing different activities in a coherent design for the attainment of specific objectives. The aim of any malaria control strategy is to prevent mortality and to reduce morbidity and the social and economic losses due to the disease. In areas where the malaria transmission has been interrupted, the goal is to avoid the re-introduction of malaria. Since the malaria situation is heterogeneous throughout the world, in the epidemiological, ecological, social and operational determinants of the disease, no single prescription can be made for the control in all countries. On the contrary, each country's circumstances influence the organization of practicable programmes able to identify local problems and priorities and to design and implement appropriate interventions. The possibility of implementing cost-effective and sustainable control measures depends on local analysis by competent national programmes for disease control.

The vastly expanded knowledge of the malarial disease and its control over the years has provided a basis for formulating a global strategy for malaria control (WHO, 1993)[1]. The Strategy does not propose a single solution but gives broad lines of approach to achieve the common aim of preventing mortality and reducing morbidity and social and economic loss due to disease through the progressive improvement and strengthening of local and national capabilities. As stated in the executive summary of the Global Strategy, "the approaches are to be adapted by the countries concerned according to the structures of their health systems and existing control operations, their resources and a realistic assessment of control needs and risk factors".

In the present chapter an outline is given of the application of the principles of the Global Strategy for malaria control in Africa South of the Sahara, where over 90 per cent of the world's malaria cases and deaths are concentrated, and where the authors have wider personal experience. While some parts of the text under situation analysis and constraints are more specific for the African context, the technical approach outlined in this chapter may provide practical and useful guidance for health planners interested in malaria control in other countries affected by malaria outside the African Continent.

Extent of the problem

Malaria is one of the most serious health problem facing the countries of Africa and a major obstacle to their sustainable social and economic development. Africa

south of the Sahara has the highest levels of malaria endemicity in the world and unlike other areas of the world where malaria is endemic, *Plasmodium falciparum*, which causes the most serious morbidity and mortality, is the predominant parasite. In this region it is estimated that between 270 and 480 million clinical malaria cases occur every year, of which about 140 to 280 million occur in children less that 5 years old (WHO, 1994a)[2].

In highly endemic areas severe morbidity and mortality are concentrated in the most vulnerable groups, pregnant women and young children. The disease causes anaemia, increases vulnerability to other diseases and contributes to the widespread problems of malnutrition. According to recent studies, malaria is responsible for about 20% to 30% of the infant mortality, and one study in the Gambia concluded that malaria mortality rate may be as high as 6.3 per 1000 per year in infants (4% of all infant deaths) and 10.7 per 1000 per year in children 1-4 years old (one-quarter of deaths in that age group).

In Africa malaria is responsible for about 10% of hospital admissions and 20-30% of outpatient consultations. Among cases referred to hospitals with severe malaria, case fatality rates of 10-30% have been noted, and in rural areas with little access to adequate treatment these rates are even higher. Taking into account the above morbidity estimates, one could expect malaria mortality in the order of 1.4 to 2.8 million annually, of which about 1 million deaths in children below the age of 5 years (WHO, 1994a)[2].

Chloroquine-resistant *P. falciparum* spread over almost all of tropical Africa in the 1980s. During recent years, high levels of resistance to chloroquine have become common in East Africa and in some countries (Malawi and Zaire) this has been accompanied by increasing incidence of severe malaria. Research results from Malawi and Kenya suggest that the prevalence of anaemia in very young children may be increasing in areas where the routine treatment of malaria is only partially effective due to drug resistance (Keuter *et al.,* 1992; Wirima *et al.,* 1993)[3, 4]. Recent studies in Western and Central Africa (Burkina Faso, Benin and Cameroon) seem to indicate a levelling off of chloroquine resistance after progressive increase in the 1980s (Chippaux *et al.,* 1990; Brasseur *et al.,* 1992; Louis *et al.,* 1992; Del Nero *et al.,* 1993; Guiguemde *et al.,* 1994)[5, 6, 7, 8, 9], but these studies did not investigate the effects of drug resistance on prevalence of anaemia.

The knowledge on health-seeking behaviour and therapy practices outside the general health services are limited, but many communities especially in rural remote areas lack adequate knowledge of the causes of malaria and methods for its control. The tendency of individuals to self-medication with incomplete dosages of antimalarial drugs drains the scarce family resources into ineffective treatment. In some cases commercial adulteration of antimalarial drugs from illegal private drug sellers also contributes to ineffective treatment and persistent morbidity.

The number of foci of intense malaria transmission have increased due to different reasons like climatic aberrations which produced epidemics in highlands and desert-fringe areas or due to population movements into malarious areas to claim land, seek wealth or escape civil disturbances and or war (Botswana, Burundi, Ethiopia, Kenya, Madagascar, Rwanda, Sudan, Swaziland, Zaire and Zambia). In certain circumstances, large-scale agricultural development projects increased malaria distribution due to attraction of large groups of non-immune labourers. The unplanned growth of peri-urban settlements with overcrowded populations near to major areas of vector breeding places creates the favourable conditions for the increase of urban malaria.

The disease is of particular significance for women as a leading cause of morbidity and mortality among the pregnant women. The negative economic, psychological and physical impacts of the disease fall especially on women who, besides suffering the debilitating effects of the disease are in most cases the primary care-givers to family members. Where women are also the economic mainstay of the family and community, this means the loss of valuable productive time for the woman and reduced resources for her family. Recent studies has shown that under-privileged women, weighed down by domestic chores, do not readily attend

clinics at some distance from home, and therefore do not have access to early diagnosis and appropriate treatment of malaria (USAID, 1994)[10].

Malaria undermines the health and welfare of families, and, debilitating the active population, strains both countries' and people's scarce resources by excessive public health costs, low productivity and impaired growth. The disease is one of the commonest cause of school absenteeism and in young adults is also one of the most frequent diseases, striking at the time of year when agricultural work is at is height. In 1987, the estimated annual direct and indirect cost of malaria in Africa was US$ 800 million and the projected figure for 1995 exceeds US$ 1800 million.

Major constraints

The factors responsible for the maintenance of the current malaria situation and for the recent increase in both incidence and case fatality rates of malaria in Africa are complex and encompass all the ecological, economic and social determinants of the disease.

The most striking feature of the disease in tropical Africa is its high endemicity, with only minor seasonal or annual changes in most areas, which is attributable to the almost ubiquitous presence of three of the most efficient vectors of malaria in the world, *Anopheles gambiae*, *An. arabiensis* and *An. funestus*. Recently ecologic, demographic and meteorological factors have led to serious exacerbations of malaria transmission in several countries, with epidemics causing severe morbidity and mortality also in the adult populations. In addition to these biological factors, the spread of parasite resistance to chloroquine and other antimalarial drugs are some of the major challenges to control programmes in Africa for the coming years.

The government's commitment to providing health care is reflected in the expenditure on health in percentage of Gross Domestic Product (GDP). In many African countries, recurrent expenditure on health often has the third place with around 20% of total recurrent expenditure, after the education and defence sectors, which often account for 25-35% each. The investment budget is often financed with foreign contributions and may be subject to strong variations from one year to another. In many African countries the proportion of health expenditure within the total governmental expenditure tended to diminish during the 1980s, but has stabilized at a low level in the early 1990s.

The capacity of governments to provide adequate malaria control through the general health services is limited due to inadequate financial resources, and the situation is worsening as a result of international economic forces. This has made it impossible for them to increase health services, or health service coverage, to any great extent and to implement an effective national health development plan. In many countries public expenditure in the health sector is heavily skewed towards high-cost hospital services that disproportionately benefit the urban population. The distribution of human resources and facilities is grossly unequal to that of the population, with low populated rural areas nearly always being undeserved.

The implications of the rapid expansion of the profit-making private health sector in Africa are quite uncertain. While in the industrialized countries, prices of drugs and medical services are regulated in an interaction of public and private interventions, insurance and social assistance systems, in African countries often the absence of regulations results in a weakening of public services and increasing use of the profit-making private sector. These developments are often accompanied by aggravation of the already inequitable distribution of health services between urban and rural areas.

The elaboration and application of a national malaria drug policy is still needed by most countries as one of the primary steps for the implementation of malaria control programmes. In several countries the malaria drug policy is not well defined within the framework of the Essential Drug Programme and of the Primary Health Care System and the national drug regulatory authorities are not supported by legislation and appropriate enforcement. New drugs, like artemisinin and its derivatives,

are being marketed in some African countries, without quality assurance prior to their distribution and in spite of no therapeutic advantage compared to the less expensive and still effective quinine for the treatment of malaria. The overall administration of supply, storage and distribution system of antimalarial drugs needs improvement in most countries.

Historically the African countries did not have well developed malaria control programmes, since the majority of them were not included in the efforts of the global malaria eradication programme of the 1950s to end the transmission of the infection. At present most national programmes are still in their planning or early implementation phase with limited infrastructure and few trained personnel knowledgeable in malaria epidemiology and management of malaria control. The need for effective information systems for monitoring and evaluation of control programmes is widely recognized, but their effectiveness is very often limited due to insufficient personnel, lack of timeliness or relevance of data and by communication problems. The situation is especially critical in countries with epidemic-prone areas, where epidemiological surveillance system are important for early detection, containment and prevention of malaria outbreaks.

The quality of case management of malaria in the health services is often inadequate due to the poor training of health workers, the absence of refresher training and of appropriate supervision. Other groups who need appropriate education include those who sell drugs officially or unofficially, private practitioners of traditional and modern medicine. They should be furnished with understandable information on the use of antimalarial drugs and the importance of ensuring that patients take the full curative dose and seek immediate competent care in the event of continued illness.

Effective coordination within different levels and sections of the health system and with the responsible officials in fields such as economic and development planning, agriculture and education is still inadequate in most countries. Institutions involved in development projects, especially forestry, water and soil conservation, irrigated agriculture, water resources development schemes, road construction etc. can bring about major ecological changes that may lead to increased malaria transmission. Very often the social, economic and environmental problems posed by malaria exceed the jurisdiction and capabilities of ministries of health and there is no legislation to bring about inter-sectoral collaboration.

In the majority of countries, community participation as a basic principle of primary health care, is still mostly taken to mean the mobilization of community resources to support an externally determined health programme or activity. Community involvement in health development, in the context of the political structure and economic situation of the country, is still a long way off (WHO, 1991)[11].

Perspective for control

It is possible to reduce the morbidity and mortality associated with malaria to acceptable low levels since malaria is a curable disease. The vastly expanded knowledge of the disease and its control acquired over the years has provided a basis for the formulation of the Global Malaria Control Strategy[1], which was endorsed by Ministerial Conference in Amsterdam in 1992, by the World Health Assembly in 1993 and by the General Assembly of United Nations in 1994. The Global Strategy calls for WHO to reinforce its leadership role in malaria control and for the strengthening of the coordination between UN agencies in the field, as recommended by the Economic and Social Council (ECOSOC) of the United Nations.

The objectives of the Global Malaria Control Strategy are to prevent mortality and to reduce morbidity and the social and economic losses due to the disease through the progressive improvement and strengthening of local and national capabilities. Since the malaria problem presents wide variations and each country's circumstances will influence the organization of practical control activities, there is a need to develop local competence to identify problems and priorities and to de-

sign and implement appropriate interventions. The Strategy underlines four basic technical elements to be the essential lines of action of each malaria control programme:

- to provide early diagnosis and prompt treatment;
- to plan and implement selective and sustainable preventive measures, including vector control;
- to detect early, contain or prevent epidemics; and
- to strengthen local capacities in basic and applied research to permit and promote the regular assessment of a country's malaria situation, in particular the ecological, social and economic determinants of the disease.

It is essential that there is a core of malaria expertise within the health ministry to assist in planning, implementation and evaluating effective and sustainable malaria control in collaboration with the general health services.

Disease management

Early diagnosis and prompt treatment services are a basic right of the affected populations, and need to be provided in all malaria situations. Populations at special risk of malaria must be identified and specifically defined so that diagnosis and treatment facilities can be focused and prompt management of the disease ensured. Children and pregnant women, on whom malaria has its greatest impact are especially important.

Given the economic difficulties of recent years, which continued to have adverse effects on health development, the priority should be to implement malaria control mainly through the general health services. Due to the limited accessibility of the population to appropriate care, especially in rural areas, disease management may need to be extended beyond the coverage of existing general health services.

The elaboration and application of a national malaria drug policy are needed by all countries since they are the primary step in the implementation of malaria control programmes (WHO, 1994b)[12]. A malaria drug policy developed within the framework of the Essential Drug Programme and the Primary Health Care System is an essential component of the National Drug Policy. The drug policy for antimalarial drugs should be developed by the Ministers of Health in collaboration with other government officials responsible of finance, education and industry, which have significant influence on drug procurement, use and manufacture. A national drug regulatory authority should be established, with interrelations with the organizations responsible for drug procurement in the public and private sectors and the standing committee on drug selection.

The drug policy should take into consideration the epidemiological factors that affect the aims of therapy, such as the geographical distribution of the parasite and its degree of resistance to drugs; the characteristics of the health services, the training and experience of the available personnel, the levels of the health service at which treatment with different drugs will be offered, and the risks and benefits of different drug regimens, compliance with them and their costs. Health ministries should ensure that all health care providers, whether public or private, are fully cognizant of ministry policies and their rationale.

Quality assurance of drugs, as embodied in product development, good manufacturing practice and subsequent monitoring of quality throughout the distribution chain to utilization, is a crucial element. Priority should be given on knowledge of and confidence in the origin of the products, and ensuring that drugs are purchased directly from known manufacturers, their duly accredited agents or recognized international agencies known to apply high standards in selecting their suppliers.

The success of the disease management programme is dependent upon the efficient administration of supply, storage and distribution system of antimalarial drugs. Government intervention is necessary to ensure the availability of drugs to the end user. Efficient management of stocks is also necessary to eliminate waste and to ensure continuous supply.

At a national level a malaria control poli-

cy defining the national objectives and organizational responsibilities for malaria control must be formulated early in the process of programme planning or reorientation. A plan for the implementation of this policy is essential, which with regard to disease management, should focus on: improving diagnosis and treatment, especially for most vulnerable population groups at risk of severe and complicated malaria, strengthening of laboratory services which should become related to patient care and ensuring that health care providers outside the health services become partners of the general health services.

Very limited human resources are available to improve disease management at the community level, especially in remote rural areas with poor accessibility to the general health services. At the community level, especially in women's groups and among schoolchildren, education enhances awareness and skills, empowering the people to participate in malaria control.

Disease prevention

Prevention of malarial disease encompasses a variety of measures that protect against infection or against the development of disease. Selective vector control include the use of chemical insecticides and biological agents and environmental management. Since proper use of insecticides for intradomiciliary residual spraying is a complex matter, involving considerable expense and demanding sustained application, it should be considered in areas where it will be both cost-effective and where environmental and social conditions, as well as the development of services for disease management, will allow the gains to be maintained.

While it is advocated that early diagnosis and treatment should be provided in all malarious areas irrespective of their levels of malaria endemicity, on the other hand, the application of intradomiciliary spraying should be done in a selective manner on the basis of the local epidemiological situation. Accordingly, existing national malaria control programmes need to be reoriented towards the concept of selective vector control which requires the stratification of the malarious areas into various categories for prioritization and selection of certain areas for spraying. Because of the dynamic changes in malaria transmission and resulting epidemiological situations, the planning process for vector control demands continuous knowledge of the local conditions. Since the timing for residual spraying is also dictated by prevailing local situations, it is extremely critical that the necessary amount and type of insecticides and spraying equipment are maintained in stock for rapid deployment in the event of emergencies.

Environmental management to reduce or eliminate mosquito breeding places should receive more attention by local communities for collective protection and be incorporated into the planning of development projects, including agriculture, water supply and other relevant activities. Budgetary provision needs to be made for the activities required, including maintenance, and national and international bodies supporting development activities should be aware of the need for careful environmental planning and management.

The use of personal protection methods by the population should be encouraged, and recent development such as insecticide-impregnated bednets with pyrethroid insecticides have been well received and have shown to give protection in some areas of Africa. Immunization against malaria may become possible in the future. Although some vaccines have been tested in the field, they are still at an early stage of development. Even when available for utilization, it is expected that they will be applied not as the sole solution to the malaria problem, but as a component of strategies that include existing and other tools.

Pregnant women require special attention. Depending on the local intensity of transmission pregnant women may be at increased risk of severe malarial illness or impaired fetal development, due to maternal anaemia and poor nutrient transfer across the placenta. Historically health services have focused on the prevention of malaria in pregnancy rather than on treatment, with chemoprophylaxis delivered in antenatal clinics. In most countries

compliance has never been adequate, and chloroquine resistance is making it increasingly difficult to define effective and acceptable regimens. Chemoprophylaxis remains desirable for pregnant women living in areas where transmission is very intense, but in some areas it may be necessary to replace chemoprophylaxis by prompt treatment of clinical episodes or periodic treatments during pregnancy (WHO, 1994b)[12].

Information system

In most countries there is shortage of people knowledgeable in epidemiology of malaria and in the planning and management of its control. The quality of care provided by the peripheral health services is often inadequate in terms of diagnosis and correct treatment. An important activity is therefore to build up malaria training capacity as part of the effort to equip all levels of the health services with competence to control communicable diseases. Trainers should include a national core group to conduct and coordinate training in accordance with national malaria policy, people with skills in malariology and educational methodologies and people in related sectors such as agriculture, engineering and health education.

For safe, effective and prudent use of drugs, relevant and reliable information should be available. Promoting rational prescribing in the private sector is notoriously difficult, as the prescriber are influenced by many different factors, including patient demand, drug advertising and drug quality. Appropriate drug information is cost effective in having drugs that are used properly and decreasing inappropriate drug use.

There is an urgent need to strengthen the capacity for early detection of epidemics. Areas prone to epidemics can be identified and local health services have a major role in reporting suspicious increase in the number of patients with fever. Contingency plans should also be worked out according to the most probable risk situations, and resources that could be rapidly mobilized should be identified. A basic preparedness measure in epidemic-prone countries is to establish a central reserve of drugs, insecticides and spraying equipment that should be permanently maintained for rapid deployment.

An appropriate epidemiological information system is an essential part of a malaria control programme, since the assessment and analysis of local malaria problems are a prerequisite for embarking on any control activity. The regular assessment of the malaria situation will allow the forecasting or early detection of epidemics, definition of risk groups, and identification of problems in programme implementation.

The policy of decentralization which is being followed by many African countries, also involves decentralization of the malaria programmes to enable local authorities to respond to the needs of individuals, households and communities promptly and appropriately. Decentralization entails the transfer of responsibilities, resources and knowledge from the central level to the local authorities and needs profound investments in local capacity building. Information systems should also be decentralized and it is important that personnel in the periphery, such as district medical officers, are trained to analyze epidemiological data, provided with the necessary technical resources and adequately supported by staff at central level.

Resources

The allocation of funds by Ministries of Health for malaria control varies from one country to another, primary due to economic considerations. Even though malaria is recognized as major health problem hindering the economic development, the scarcity of funds has prevented most Governments from launching effective programmes without external assistance. Many countries, however, have shown increased commitment towards strengthening malaria control programmes by supplementing external assistance and mobilizing additional resources, e.g. Ethiopia (US$ 8-11 million for 1994), Ghana (US$ 3 million), Namibia (US$ 2-4 million) and Zimbabwe (US$ 3 million).

To overcome the severe shortage of drugs and to ensure continuous supply

and availability of drugs there is a tendency in many countries to apt for cost-sharing or recovering systems. The performance of these systems in term of recovering the costs and using them for buying new supplies is variable. These efforts may be linked to essential drug policies which aim to improve the availability of essential, affordable drugs, to promote rational use of drugs, and to contain costs.

Non governmental organizations (NGOs), including religious institutions often run services benefiting the most underprivileged populations. Primary health services are re-appearing as community health centres, with physicians working in big villages and private or community-based pharmacies. The creation of central facilities for drug procurement and importation for the private sector (profit-making or not) with the objective of importing essential drugs at low price are recent initiatives with important positive implications for malaria control.

At present the majority of African countries are not involved in vector control activities, and rely on disease management through the health services and community-based interventions. The cost of malaria in most of these countries represents an average of 10% of the total public expenditure on health, which corresponds primarily to the payment of the salaries of health personnel and the limited supply of antimalarial drugs. A significant number of malaria patients obtain treatment outside the formal health services and the expenditure by the population on malaria treatment exceeds that of the public services. In addition to the cost of antimalarial drugs, it is also estimated that the population spends at least US$ 2.0 per person per year on personal protection against mosquitoes.

An increasing number of countries have prepared detailed and comprehensive plans of action for malaria control based on the principles of the Global Strategy. The planned activities include:

- training of health workers at all levels of the health care system on management of malaria disease;
- strengthening of diagnostic facilities for the management of severe cases and treatment failures;
- training of community health agents and drug vendors at the periphery on malaria diagnosis, treatment and correct prescription;
- development and use of health education materials targeted at the health workers, mothers and the general population;
- introduction of insecticide-impregnated bednets in limited situations;
- strengthening of the routine epidemiological information system for assessments of local trends of malaria, the efficacy of antimalarial treatment, and the detections of malaria outbreaks and epidemics.

The cost of this "minimum package" of basic activities is estimated to be US$ 300,000 per year per country, which in most cases in Africa cannot be covered by the national budgets and will require external funding.

In recent years an increasing number of malaria epidemics have been reported in several African countries. Several of these epidemic-prone countries finance their respective national control programmes, including the costs of insecticides, from their national budgets, but more resources are needed to improve the epidemiological surveillance system and to strengthen the capabilities in vector control. It is estimated that an additional financial support of US$ 1 million per year would be required for those with epidemic-prone areas in order to carry out a comprehensive, effective and sustainable programme, which includes selective vector control.

It can be estimated that the external investments in malaria control for this decade should be approximately US$ 14 million per year for the implementation of basic programme activities related to disease management and an additional US$ 12 million per year in 12 epidemic countries of Africa for comprehensive control activities, and thus a total of US$ 26 million per year is needed for endemic countries in the region. This financial support to Africa could be secured through bilateral or multilateral arrangements. WHO will provide, within the limits of its resources, technical support as required.

Conclusions

To prevent mortality and reduce morbidity as well as the social and economic losses due to malaria in Africa, Governments should coordinate and promote the following recommendations for action.

1. Malaria control should receive long-term national political and financial commitment, with proper resource allocation in the context of national health care and development planning;
2. There is a need to establish an appropriate malaria drug policy within the framework of the national drug policy, to provide drugs that are of acceptable quality at an affordable price and to ensure that they are used rationally;
3. Local capacity-building is a prerequisite for achieving effective and sustainable malaria control and special attention should be given to, and resources for, training and human resource development at all levels;
4. More attention should be given to community's perceptions and health-seeking behaviour and to the possibility of wider involvement of the community in the determination of priorities and in the allocation of the scarce health resources for malaria control;
5. It is essential that there is a core of malaria expertise at the national and regional level to assist in planning, implementation and evaluation malaria control as part of the national development plan. The training of entomologists and epidemiologists to assist in reorienting and guiding programmes, planning and implementing selective vector control;
6. Capacity and services for disease control and prevention should be decentralized to the maximal possible extent, since local conditions will determine the most practicable way to identify priorities and plan and implement appropriate interventions. The key for effective control is competent local action;
7. Interdisciplinary and intersectoral collaboration at all levels should be prompted to ensure effective and sustainable malaria control. This may require high-level political commitment and legislation to ensure intersectoral collaboration;
8. All control programmes require operational research to address not only the efficacy of specific interventions, but also social, economic, cultural and behavioural factors that affect malaria control; national programmes and research institutes should collaborate more closely to increase the relevance of research to malaria control;
9. Additional resources for malaria control should be sought from international sources and from communities. Successful resource mobilization will require national political commitment, clear work plans and effective coordinating mechanisms to be developed within countries as a responsibility of the national authorities;
10. There is a need to establish a policy for insecticides in public health in order to protect the safety of the population and of the environment;
11. The occurrence of epidemics is on the increase in Africa and in those countries where it is a major problem it will be important to strengthen the vector control activities of the malaria control programme.

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MIGRATION AND MALARIA

R. M. Prothero

Introduction

The relationships between migration and malaria are complex. They are two-way relationships – migration affects malaria and malaria affects migration. The former is the concern of this chapter.

An up-to-date comprehensive study of migration/malaria relationships has still to be written, since three decades have passed since the publication of *Migrants and malaria* (Prothero, 1965) (US edition, Prothero, 1968)[1]. This was concerned exclusively with Africa where pilot malaria eradication projects in the continent were being affected negatively by the movements of people (Prothero, 1961)[2]. At this time there were continuing but diminishing hopes that global malaria eradication was possible.

During the last three decades there have been changes in migration and in malaria in Africa and in other malarious parts of the world. Eradication has been replaced by control which requires the ongoing application of anti-malaria measures, and *falciparum* resistance to chloroquine (and in some places to other drugs) has become widespread. The nature and, more particularly, the scale of migration has changed substantially. Evidence emphasising and exemplifying the importance of malaria/migration relationships has increased, contributed to by bio-medical and social scientists. There has been an increasing recognition of this importance by the former group, a recognition which in the past was limited and which is still not as satisfactory as it should be. In the malaria equation – parasite/vector/people – parasite and vector have received much greater attention than have people. The everyday aspects of people and their lives are of fundamental importance in malaria and its control, among these aspects movements of many kinds are of great significance.

The significance of migration for malaria may be summed up simply as follows.

1. Movements of people in various ways contribute to the transmission of malaria infections.
2. Programmes for malaria control/eradication, and for the improvement of public health in general, are hindered when applied to populations which are in whole or in part mobile. Mobile people are more difficult to access and to contact, and the costs of doing so are much greater than when people are relatively sedentary.

In recent decades (Prothero, 1977)[3]:

1. there is greater movement of people than there was in the past, for with the rapid growth of population there are now more people to move;
2. the rapid growth of population exerts pressures on resources with major redistributions of people as they seek to improve their economic circumstances;
3. political and environmental disasters may

force people to move, with increased movements of refugees and victims of drought, famine earthquakes etc.;
4. the means of movement are greatly increased, so that movements can take place over much greater distances and in much less time than in the past with heightened risks of disease transmission and of imported diseases.

The nature of migration

Migration is not uniform; there are many different types. Movements of people vary in their causes and consequences, and in their spatial and temporal patterns (where and when movements take place and distances covered). All of these may be of importance in their impacts on malaria; particularly spatial and temporal patterns of movement about which remarkably little is appreciated and known.

It is important to distinguish between:
1. movements which involve a definitive and permanent change of place of residence between one place and another, which may be termed *migration*;
2. movements which involve an absence from a place of residence for a period of time followed by return to it, which may be termed *circulation*.

The spatial and temporal characteristics and patterns of these two major categories vary considerably.

Migration may occur in a regular fashion, movements taking place after due thought and planning for a new place of residence. Or it may occur in irregular fashion, taking place relatively precipitately as a consequence of unforeseen factors (war, environmental catastrophe, famine). Irregular migration is likely to be more significant in its effects on malaria.

Circulation may occur for limited periods of time (daily), for longer periods which may extend for weeks, months or even years, or for periods which are defined by the length of seasons (winter/summer, wet/dry). All of these can have significant effects on malaria.

Both migration and circulation occur within rural areas (rural-rural), between rural and urban areas (rural-urban, urban-rural), and within urban areas (urban-urban).

Also, both migration and circulation take place within countries (internal) and between countries (international), and increasingly between continents. In many parts of the world, where international boundaries may not be recognized and are easily crossed, it may be difficult to distinguish between internal and international movements. This has important implications for malaria transmission and for the design of control programmes which require international cooperation and coordination (see later discussion).

To begin to understand relationships between migration and malaria it is as important to recognize the variations in movements outlined above as it is to recognize the various species of malaria parasites and vectors. It must be realised that movements of people, in whatever forms and under whatever circumstances they occur, are difficult and for the most part impossible to control. Furthermore, they cannot be subject to experiment. It is therefore essential that anti-malaria operations and projects for public health improvements should be designed to work within the constraints imposed by the movements of people and that these constraints are clearly understood.

Population movements and malaria (Tables 1a and 1b)

Through movements people are exposed to malaria risks in a variety of ways (Prothero, 1977)[3].

1. As they move between and through different ecological zones; for example, between arid or high altitude areas which are malaria-free and irrigated or low altitude areas which are malarious.
2. Through exposure of people with little or no immunity to infected persons, particularly when strains of malaria are drug-resistant.
3. Through physical and psychological stresses (fatigue, undernutrition/malnutrition, problems of mental adjustment in new environments) which reduce resistance to infection.

	Time					
	Circulation			Migration		
Space	Daily	Periodic	Seasonal	Long term	Irregular	Regular
Rural-rural	Cultivating Gathering (firewood, water)*	Hunting*	Pastoralism*°	Labouring*°	Gathering*°	Planned settlement*°
Rural-urban	Commuting*	Pilgrimage*°a	Labouring*	Labouring*°a	Environmental victims*°a	Labouring°a
Urban-rural	Cultivating*	Trading[a]	Labouring*	Trading*°	Refugees*°a	Visiting/ retirement*
Urban-urban	Intra-urban commuting°a	Pilgrimage*°a	Trading[a]	Official commercial°	Refugees°	Change of residence°

Malaria risks
* Exposure to malaria from movement between different ecological zones.
° Exposure to malaria from movement involving contacts between different groups of people.
[a] Physical stress (fatigue, undernutrition/malnutrition) and physiological stress (problems of adjustment)
Note: any movement may affect programmes for control and/or eradication.

Table 1a. Populations movements and malaria in Africa (with examples of movements and risks (Prothero, 1977)[3].

	Time				
	Daily	Periodic	Long term		Migration
Space	Return after or late	Overnight to < 1 week	1 week to < 1 month	1 month to < 1 year	1 year or more
Rural village to foothills	Hunting (frogs)* Collecting (bamboo shoots, firewood)*		Cultivating (maize, cotton)*		
Rural village to upland forest		Hunting (game)*	Logging, Hunting (khiat laeo)* Collecting (honey)*	Forest plantation* Construction (road, dam)*	Mining (ores)*°
Rural village to Burma, Laos, Cambodia	Smuggling (goods)*°	Labour (sawing logs)* Visiting(kin)°	Trading (cattle, goods)*° Labouring (cowhands)*°	Cultivating (opium)*° Construction (temples houses)*° Mining (gems)*°	Mining (ores)*
Burma, Laos to forest settlement in Thailand		Visiting (kin)°	Labouring (cowhands)*°	Forest plantation*° Labouring (farm)*° Refugees*°	Mining (ores)° Refugees°
Burma, Laos to Thai border towns	Trading (goods)°	Visiting (kin)°		Housemaids (shop/ restaurant helpers)°	

Malaria risks: see legend in table 1a.

Table 1b. Circular mobility in Northern Thailand and activities associated with malaria transmission (Singhanetra-Renard, 1993)[4].

Some examples of these risks in relation to the variety of movements outlined previously are as follows.

1. The daily or weekly circulation of hunters, gatherers and farmers may involve movements between areas which differ ecologically in minor respects but still increase contacts with vector-breeding habitats (Singhanetra-Renard, 1993)[5]. Such movements on forest fringes, particularly in South-East Asia, have given rise to what is now identified as "forest malaria" (Sharma and Kondrashin, 1991)[5].
2. Seasonal circulation may involve farmers during times of maximum agricultural activity (in the wet season) in movements from relatively vector-free villages to temporary dwellings on farmland which are foci of vector activity. Or seasonal circulation may take migrant labourers with little or no immunity from higher-altitude, malaria-free areas to lower-lying malarious areas where they suffer severe infection.
3. In the case of regular migration, problems may arise for those who make planned and organized moves from rural to urban areas and settle there for long periods of time. Since these urban areas are relatively malaria-free those who have moved to them will in time have reduced immunity. But it is common to make short visits back to places of rural origin and at these times they may be exposed to the risk of malaria infection.
4. Irregular migration, caused by political pressures or by environmental castastrophe, exposes people to physical and psychological stresses and consequently increases their susceptibility to malaria and to other infections (Prothero, 1994)[6].

There are contemporary movements of large numbers of people, significant in various ways for the transmission of malaria and affecting measures for malaria control, to which particular attention should be directed.

1. Movements which are the result of political disruption and environmental catastrophe, especially the large-scale movements of refugees.
2. Movements associated with the resettlement of population for political and for economic reasons.
3. Movements from rural into urban areas.

Refugees in large numbers have exacerbated already complex malaria situations – in north-east Africa as a consequence of Ethiopia/Somalia conflict, in south-west Asia as a consequence of war in Afghanistan, in south-east Asia from conflict in Vietnam and Cambodia, and in Central America. The relatively precipitate and chaotic nature of refugee movements makes forward planning virtually impossible. Higher incidence of malaria is recorded in those parts of countries most seriously disrupted, and it is a major problem in refugee camps. Measures for malaria control are restricted and sometimes impossible to effect; control of vector breeding has not proved particularly successful in circumstances where populations are transient. When vector control is not possible protection with drugs should be reserved for non-immune and high-risk groups (malnourished children and pregnant women).

Refugee movements have played a significant role in the spread of drug-resistant *falciparum* malaria in South East Asia in the last three decades, particularly from war in Vietnam and Cambodia and from disturbed conditions on the Thai-Myanmar border (Verdrager, 1986)[7]. From a South East Asian focus chloroquine-resistant *falciparum* has spread into north-east India and from there south and west into other parts of the sub-continent (Payne, 1987)[8]. This spread is further referred to later in this paper.

The movements of people for resettlement in "frontier/pioneer" areas are particularly favourable for increased malaria transmission.

Forced resettlement in Ethiopia in the 1980s brought people from the traditionally preferred areas of occupation at altitudes above 2000 m, generally malaria-free but where malaria epidemics occur periodically, to lowlands where the dis-

ease is endemic. Settlers lacking immunity were at risk to high morbidity and mortality from malaria and also from other infectious diseases (sleeping sickness, river blindness and yellow fever). Malaria risks were increased in agricultural settlements with irrigation, and seasonal movements of migrant labour also contributed to the spread and maintenance of transmission (Kloos, 1990)[9].

Transmigration policies in Indonesia have moved people from the densely-populated and relatively malaria-free inner islands of Java and Bali to outer islands (Sumatra, Kalimantan and Sulawesi) which are malarious and where health service to deal with this and other diseases are inadequate. The success of malaria control on the inner islands has meant that migrants from these have low levels of immunity, but in the resettlement islands they are exposed to endemic malaria with a greater variety of vectors in some islands (Sulawesi and Irian Jaya) than in others. Water developments (irrigation, rice cultivation, fish culture) increase the habitats for vector breeding (Abisudjak and Kotanegara, 1989)[10].

Outstandingly in the last two decades the Amazon basin has witnessed a resurgence of malaria associated with frontier settlement, with a ten-fold increase in the number of reported cases to over 600,000 in 1992, more than a half of all those recorded in the Americas. The physical environment favours vector breeding, but this has been enhanced by settlement construction, road building and mining which extend breeding sites for *An darlingi*, the major vector (Coimbra, 1988; Sawyer, 1993)[11, 12]. Movements of population have been important in malaria transmission and have reduced the effectiveness of government programmes for malaria control.

Many of the migrants originate in the dry north east and in the southern more temperate parts of Brazil, both for the most part malaria-free, and having no immunity they suffer severely when they arrive in the Amazon region. In reverse of this situation, when infected persons from the Amazon have moved south they have been the source of minor localized outbreaks of malaria in Parana which is generally free of malaria but where there are mosquitoes which can transmit the disease (Cruz Marques, 1987)[13].

Temporary dwellings cannot be protected with insecticide spraying, in clearing land settlers are exposed to vector-breeding habitats, and poverty limits their access to measures for protection against malaria. Besides agricultural settlement malaria has increased through mining activities which increase vector-breeding sites, temporary settlements are unprotected and the continual in and out movements of miners maintain the reservoir of infection and make control virtually impossible (Vosti, 1990)[14].

Such control as can be achieved in the Amazon basin is principally through the use of residual insecticides (most commonly DDT). Anti-malarial drugs are used frequently without medical supervision and indiscriminate use contributes to the development of *falciparum* resistance, particularly to chloroquine.

Generally in the malarious parts of the world there is more effective control of the disease in urban than in rural areas, but the continuing large-scale movements of rural people to the towns threaten this control (Service, 1989)[15]. Rural migrants are infected and the makeshift temporary shanty-towns in which they live provide habitats suitable for mosquito breeding. In India *An stephensi*, which formerly bred in wells, now breeds in these urban habitats. Urban malaria, which was of marginal importance in India's malaria eradication programme in the 1960s, is now well established in many Indian cities, with about a third of reported cases occurring in urban areas. Circumstances vary, in the early 1990s malaria cases were fewer in Delhi and Calcutta but increased in Madras and Bombay. Manaus, the major city of the Brazilian Amazon, experienced several epidemic outbreaks in the 1980s on its peripheries where the shanty towns are concentrated.

Specific malaria projects

The previous discussion has outlined in general terms the relationships between malaria and different forms of population

mobility, and the need for these to be recognized and understood. Evidence in greater detail of these relationships is limited but some indication must be given of their nature and how they may be investigated.

Garki, Northern Nigeria

The WHO project at Garki, Northern Nigeria during the first half of the 1970s researched the epidemiology and control of malaria (using residual insecticides and drugs) in the Sudan savanna, where there are large yearly and local variations in the level of transmission (Molineaux and Gramiccia, 1980)[16]. It concentrated particularly on the measurement of entomological, parasitological and sero-immunological variables and their relationships. Demographic data were collected for the de facto population in the project area, recording birth, deaths, arrivals and departures. All data were recorded longitudinally.

The population was shown to be relatively mobile, with marked variations in patterns of movement seasonally and from one year to another, with some differences between one village and another. However, only two types of movement were measured – movement in and out of villages and short-term absences from villages – and their geographical distribution was not recorded. At least two other types of movement which were recognized as important – short-term visits to villages (which were substantial and variable) and movements of semi-nomadic pastoralists – were not measured and therefore no assessment could be made of their influence. Little account was taken of the fact that the project was undertaken during a period of intense drought which substantially affected many of the movements.

Overall it was concluded that there was no difference in respect of malaria either between those who left and those who stayed in villages, or between those who came and those who were already there and it was concluded

> "It is very unlikely that the mobility of the human population, which was relatively pronounced, was the main cause of the maintenance of transmission".

While this broad statement may be correct the more precise effects of mobility remained unknown and unproven. Data were not complete and certainly not all types of movement occurring in the project area were identified. Despite the range of medical and entomological personnel in the project team there was no social scientist specifically concerned with the study of the mobility and other socio-economic characteristics of the population. A recommendation made at the planning stage of the project that such a person should be included was unfortunately not accepted (Prothero, 1977)[3].

Naya Basin, Colombia

A more recent study of malaria and mobility in the Naya river basin of Colombia presents evidence of more effective interdisciplinary involvement of entomology, malariology and social science (Sevilla-Casas, 1993)[17]. An initial geographical survey defined ecological zones and the types and locations of settlements from which a cluster sample was drawn to provide households which were surveyed for composition and structure, mobility history, occupation and malaria experience during the previous year. A smaller representative sample provided more detail on the ethnography of village life, circulation movements and malaria prevalence.

At a macro level data on endemicity and mobility (associated with agriculture, logging and trade) identified that the malaria risk was greatest in the delta of the Naya river into which there were movements for economic reasons at times when vector densities were high and biting most intense. This combination of high human and high vector density ensured continuous and intense transmission of predominantly *falciparum* malaria, with high prevalence among both sexes, prevalence increasing with age from adolescence onwards as the range and number of peoples' activities increased.

Micro study of the daily economic activities in two villages with high endemicity identified particular areas within the delta with greater or lesser risk of malaria transmission. People moving within the beach area of the delta were exposed to the

greatest relative risk from the high biting rates of *An albimanus* in this environment.

Two matters emerged from this study which are of practical importance for the design of more effective measures of selective prevention and of adequate treatment in malaria control programmes, particularly among populations where a high proportion are mobile.

1. The need to identify areas where the risks of infection are greatest, and to concentrate control resources on these rather than spread them more widely.
2. Routine malariological surveys had been inadequately representing the numbers of infected women.

It is known that non-immune pregnant women are particularly susceptible to malaria infection and should receive priority protection (Reuben, 1993)[18]. Furthermore, women have been neglected in the study of tropical diseases, in general and as migrants who may be exposed to these diseases (Vlassoff and Bonilla, 1994)[19].

A further study may be consulted. It reports on the influence of the movements of pilgrims on the persistence of malaria in south India (Rajagopalan *et al.*, 1986)[20].

Trans-boundary movements and malaria

Mosquito vectors and infected and non-immune persons are not restricted in their movements by state or international boundaries in any of the malarious parts of the world or in areas adjacent to them. For example, malaria was virtually eradicated from the USA during the first half of the present century, but as recently as 1986 there was an outbreak of malaria in San Diego County, California for the first time since the early 1950s. There were vectors to transmit infection and its source was infected migrant workers from Mexico (Maldonado *et al.*, 1990)[21].

Since it is difficult and almost impossible to control the reservoir of infection and transmission when there are movements of people between countries, agreements and arrangements to deal with these circumstances are essential. Without them effective control measures in one country are likely to be negated by the absence of measures in countries adjacent to it (Prothero, 1965)[1].

In Latin America the most satisfactory of these agreements is the Southern Cone Pact involving Bolivia, Brazil, Paraguay, Uraguay, Argentina and Chile. Malaria is endemic in the first three and in a small area of northern Argentina, but the greater part of Argentina, Uraguay and Chile are malaria-free. The Pact provides for the exchange of information on malaria and resources for its control. Elsewhere in the highly malarious areas of Latin America there are varying degrees of coordinated malaria control in areas adjacent to and across international boundaries. Coordination and control are limited by political inaction and in some instances by political friction, these being exacerbated by refugee movements and by clandestine and illegal movements of migrant labour. In the coca-growing countries problems are made even worse by drug-trafficking and by the attempts to control this. Overall inter-country cooperation and coordination for malaria control in Latin America are poor, though probably no worse than in most other malarious areas of the world (Prothero, 1995)[22].

Probably the present and potentially most difficult region, where population movements promote malaria transmission and drug resistance and complicate measures to counter these, is South East Asia. Here as in many other parts of the world international boundaries have little meaning for the people who live adjacent to them, and in many instances the boundaries artificially divide ethnic groups whose members are continually crossing for economic and social reasons. Many of these ethnic groups are small and are often in conflict with the central governments of the countries of which they are part. Problems of control are compounded by the fact that many of the boundaries run through mountainous and heavily-forested country which is inaccessible and where movement is difficult for the authorities (immigration, customs and health) who wish to exercise control.

Attention has recently been drawn to the "corridor" running through Myanmar,

Thailand, Cambodia, Laos, Vietnam and southern China where there is rapidly developing trade and commerce, legal and illegal, involving the movements of large numbers of people which are likely to increase in the future (Kidson, 1993)[23]. Of a total population of about 300 million, one fifth who live in the more inaccessible parts are at risk of malaria infection, with about a million cases a year, About a half of these cases are *falciparum* infections resistant to chloroquine and to other antimalarial drugs. Furthermore, most of the populations of the major vectors – *An dirus*, *An minimus* and *An maculatus* – are exophilic and exophagic and resistant to control measures.

Official border posts estimate that movements between Yunnan and Myanmar, Laos and Vietnam involve more than 10 million people in the course of a year, and at one crossing point on the Thai-Cambodian border more than 40,000 passed during one month. To these must be added unknown but probably larger numbers involved in illicit activities (gems, drugs and arms traffic) who avoid, as they can easily do, any border controls. The contribution of these movements to the transmission of malaria (and more recently of AIDS) and to the build-up and spread of multi-drug resistance cannot be estimated but is of enormous significance. Government attitudes, at least to legal economic activities, are that these should be encouraged and officials are often less concerned with their negative effects on disease spread and control.

In these difficult circumstances there is an urgent need for international cooperation and coordination within the region. Moves towards these were made at a meeting held at Kunming in November 1993 organized by the WHO and the Ministry of Health in China (Karbwang and Harinasuta, 1993)[24]. National malaria programmes need to exchange information on the most effective drugs to be used and their correct dosage, on developments in drug resistance, on vectors, and on changes in socio-economic factors (including movements) which affect all of these. Such measures are required in all other malarious parts of the world.

Besides movements affecting malaria within continents there are the increasing number of inter-continental movements which are resulting in malaria occurring in areas from which it has been eradicated. The US example was quoted earlier, and more recently in 1993 there was a localized outbreak in Singapore when infection was introduced from outside the country.

Cases of "imported malaria" occur in Europe as a consequence of the greatly increased movement of non-immune travellers from that continent, particularly tourists seeking holidays in exotic places which are malarious without adequate chemoprophylaxis. There were some 8000 reported cases in Europe in 1992 but the actual number is reckoned to be greater. In the UK in 1994 1800 people returning from malarious areas developed the disease and 11 died, the risk of fatality being much greater for those with *falciparum* infections. Deaths often occur because of the failure of travellers to declare that they have been in malarious areas, and whose illnesses have consequently been incorrectly diagnosed. Instances have also been recorded of malaria infection occurring in malaria-free areas from infected vectors being transported on aircraft.

Conclusion

Movements of people in their many different forms present major issues in the latter decades of the twentieth century at a variety of scales – global, inter- and intra-continental, national, regional and local. They are causally related to a great range of factors and similarly have wide implications – political, social, economic and, as in this chapter, medical, for malaria and for many other public health problems. Though the medical implications are being increasingly recognized, this recognition has been slow in developing and, as stated earlier, the basic facts of peoples' lives, including their movements, have not received the attention they require in planning malaria control and eradication.

The reports of the WHO Expert Committee on Malaria from the 1950s onwards have made reference to the significance of population movements for malaria transmission and for programmes for its reduc-

tion. This significance is recognized in the standard work, *Malaria: principles and practice of malariology* (MacGregor and Wernsdorfer, 1988)[25], but there is virtually no reference to it in *Malaria: waiting for the vaccine* (Targett, 1991)[26]. In the past and at the present much lip-service has been paid to this significance but too little practical action has been taken. The fact that in anti-malaria work people and their actions require the expert attention of social scientists, as do parasites and vectors the expert attentions of malariologists and entomologists respectively, has not been fully appreciated.

Advances have been made, particularly with the setting up in the late 1970s of the Socio-Economic Working Group in the World Bank/UNDP/WHO Special Programme for Research and Training in Tropical Diseases (Vlassoff, 1991)[27]. Reference is made earlier in the paper to some of the outcomes of this development (see also Ministerio da Saude, 1982; Fernando, 1984; Singer and Oya Sawyer, 1992)[28, 29, 30]. There is now wider *multi*-disciplinary involvement in the study of malaria control and eradication, but still less *inter*-disciplinary involvement than there should be. Malariologists, entomologists and social scientists still have to learn to work together more closely, and particularly for the last of these to be more accepted by their colleagues who study parasites and vectors. These will continue to be necessary even if and when more effective anti-malarial measures (drugs, vaccines and insecticides) become available.

It is hoped that what has been set out in this chapter will point up the significance of migration in its many forms in malaria transmission and in programmes for control/eradication. If social science expertise should not be available then it is even more important that malariogists and entomologists should know something of people and their movements, so that they are aware of the problems that these may create. They may then be able to adjust their activities more appropriately to matters of importance which have been neglected in the past.

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FUTURE PERSPECTIVES IN MALARIA CONTROL, INCLUDING VACCINE

H. M. Gilles

The various malaria control strategies available have been described in detail previously. They are presented diagramatically in figure 1.

Historical perspective

Environmental sanitation works, both for health purposes and agriculture, were undertaken by the early civilisations of Mesopotamia, Egypt and the Indus Valley and are mentioned by Greek, Chinese and Roman writers. They continued during the Middle Ages into modern times. Lanusi in 1717, not only proposed a plan for the drainage of marsh lands but also formulated the theory that malaria may be caused by noxious juices inoculated by insects breeding in the marshes. A well documented history is that of the Pontine marshes, eventually successfully drained during the Mussolini era.

Personal protection against insects – gnats – dates back to Herodotus (c. 484-425 B.C.), while bed-nets were used in colonial America as early as 1684 and widely adopted by the African explorers of the 19th century.

Antimalarial treatment with Quinghaosu was known to the Chinese for two millenia before Cinchona bark was discovered by the Spaniards around 1600 and advocated not only for the treatment, but also for the prophylaxis of malaria.

The importance of *socio-economic* factors in the disappearance of malaria from England was highlighted by James (1929).

The transition from global malaria eradication (1955-1979) to a strategy of malaria control (1979-1985); to the integration of malaria control as an integral part of a national primary health care system (1985) culminated, in the Ministerial Conference on Malaria in Amsterdam in 1992, which contained four basic elements:

1. to provide early diagnosis and prompt treatment
2. to plan and implement selective and sustainable preventive measures
3. to detect early, contain or prevent epidemics
4. to reassess regularly a country's malaria situation, in particular the ecological, social and economic determinants of the disease.

Early diagnosis and prompt treatment

Reduction of malaria morbidity and mortality – a comparatively modest goal when

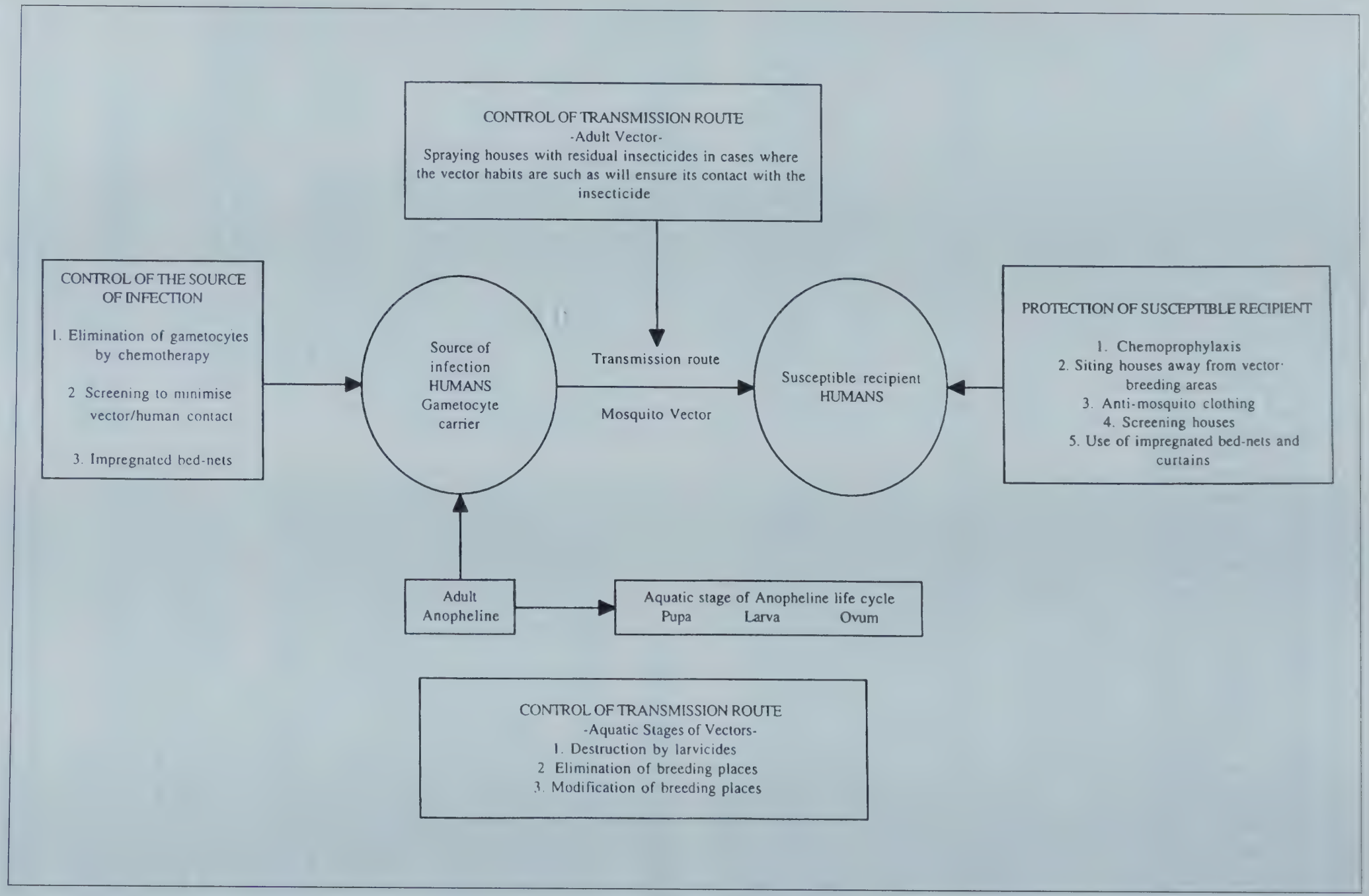
CONTROL OF TRANSMISSION ROUTE
-Adult Vector-
Spraying houses with residual insecticides in cases where the vector habits are such as will ensure its contact with the insecticide
CONTROL OF THE SOURCE OF INFECTION
1. Elimination of gametocytes by chemotherapy
2 Screening to minimise vector/human contact
3. Impregnated bed-nets
Source of infection HUMANS Gametocyte carrier
Transmission route
Mosquito Vector
Susceptible recipient HUMANS
PROTECTION OF SUSCEPTIBLE RECIPIENT
1. Chemoprophylaxis
2. Siting houses away from vector breeding areas
3. Anti-mosquito clothing
4. Screening houses
5. Use of impregnated bed-nets and curtains
Adult Anopheline
Aquatic stage of Anopheline life cycle
Pupa Larva Ovum
CONTROL OF TRANSMISSION ROUTE
-Aquatic Stages of Vectors-
1. Destruction by larvicides
2 Elimination of breeding places
3. Modification of breeding places

Fig. 1. The control of malaria.

compared with Global malaria eradication – will be the main challenge for the future, and more effective measures than we have at present within the prevailing *primary health systems*, will have to be devised.

It is generally accepted that the majority of deaths due to malaria in children, particularly in Africa, never reach a health post.

One obvious requirement for the future, would be to increase the availability and provide competent malaria disease management at the periphery, using *adequately* trained health workers, or health volunteers, to recognise and treat uncomplicated cases, or refer severe cases. In the latter context, the advent of artemesinin or quinimax suppositories could have a significant impact. One could envisage the following sequence of events – recognition of severe disease; taking a thick blood film or "dip stick" of diagnosis; administration of suppository; referral of patient to a higher level health post.

The WHO/UNICEF approach to the "*Integrated management of the sick child*" provides many obvious advantages, especially when one bears in mind the overlap between severe malaria, ARI pneumonia and meningitis.

Treatment of *P. falciparum* malaria in children, in whom the antecedent history before severe disease manifests itself is short (2 days); cannot wait and requires easy accessibility of antimalarial drugs and knowledge of their proper use. Community health workers, responsible village lay people and mothers, could all be taught to recognise and refer severe cases – at least until such time as enough recognised health posts are available within very short distances of villages and in which a constant supply of antimalarials is always readily available.

Ambulatory drug sellers and owners of grocery stores, who are the commonest sources for self-medication, could be taught to provide a full course of *reliable* antimalarials and advise mothers accordingly. Self-medication is a very common practice in most malaria endemic countries (Deming *et al.*, 1989; Louis *et al.*, 1992; Ruebush *et al.*, 1992)[1, 2, 3], every effort should be made to improve its quality, since this practice is likely to continue, or even increase.

The improvement of the diagnosis and treatment of malaria will require reorientation of existing practices to take into account the above considerations.

Reduction of man-mosquito contact

Sir Ronald Ross, in 1911, commenting on personal protection, wrote as follows "I have had experience on this point during many years and in many different countries, and have only once been infected... I always rely upon the possession of four articles, namely, a good bed net, ... a small hand net for catching mosquitoes, ... a palm leaf fan, ... and a bottle of quinine. With these articles, the chances of becoming infected are reduced enormously – especially if a little common sense and care are added to the stock".

The use of *impregnated bed-nets and/or curtains* with residual pyrethroids, e.g. permethrin and deltamethrin, is likely to increase, once their value in reducing malarial morbidity is more widely established. Collective action by communities with (possibly) government support would be needed to organise periodical regular reimpregnation of the bed-nets and ensure *sustainability*. It has been shown that the success of bed net programmes was dependent on a variety of factors – vector susceptibility to pyrethroids, high coverage of the population at risk, high malaria incidence, good community participation, high mosquito densities when people go to bed and a high prevalence of *P. falciparum* (Kroeger *et al.*, 1995; Alonso *et al.*, 1991; Lyimo *et al.*, 1991)[4, 5, 6].

Vaccination

The present concepts of malaria control envisage that primary health care is expected to assume an important role; yet the world-wide implementation of primary health care is progressing at a much slower pace than was envisaged, due to financial, administrative and technical difficulties.

The widespread resistance of anopheline vectors to insecticides; the exophilic behaviour of some of the anopheline vectors; the virtually global spread of chloroquine resistance; the increasing development of multidrug resistance of *P. falciparum*; the relative paucity of new antimalarial drugs; economic, social and behavioural factors, have significantly diminished the traditional antimalaria armamentarium. It is therefore in the context of this depressing scenario that vaccination has got to be considered.

The first significant breakthrough in the production of a malaria vaccine against *P. falciparum* was made by Patarroyo *et al.*, (1988)[7]. His multivalent synthetic vaccine gave encouraging results in human volunteers in Colombia. This prompted WHO/TDR to embark on further field trials in the Gambia, Tanzania and Thailand. Unfortunately, when tested in an area of perennial transmission in Tanzania, a 31% efficacy was reported for the "spf 66" or Patarroyo vaccine, as it is generally known (Alonso *et al.*, 1994)[8]; while in the Gambia, only a 3% efficacy for the same molecule was found (D'Alessandro *et al.*, 1995)[9]. Similar low efficacy has been recently reported from Thailand.

Vaccine development is progressing at a very fast pace and the tendency is towards producing a "cocktail" vaccine which will include a combination of multiple epitopes from different malarial antigens and from different stages of the parasite life-cycle.

Full trials with these improved vaccines will take place in the foreseeable future and the results will be awaited with great interest.

Once the efficacy of a vaccine is proven in well-designed field trials, a variety of other important questions will have to be considered, e.g. (1) how long is the duration of protection? (2) at what age should it be given in endemic malarious areas? (3) how frequently? (4) do genetic factors affect the immune response? (5) is the vaccine equally effective in various areas of the world? (6) what will the cost of the vaccine be? (7) will it protect against severe disease? (8) will resistance forms to the vaccine develop? Although most efforts are directed justifiably against *P. falciparum*, vaccines against *P. vivax* are also being developed for countries where this is the predominant parasite. They have not yet reached the stage of development that *P. falciparum* vaccines have attained.

Once an effective vaccine is found, and the efforts that are being put into accomplishing this, are in my view, highly commendable, as well as worthwhile, it is predictable that it would play a significant role in the control of malaria; not on its own, but more likely in combination with the other elements of an *integrated* malaria control strategy.

Meanwhile, our only alternative in the pursuit of this new weapon for continuing the fight against malaria, is to follow the Ulyssian call; "To strive, to seek, to find and not to yield!" (Bruce-Chwatt, 1956)[10].

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FUTURE PERSPECTIVES IN DRUG RESEARCH

P. Olliaro

Background

Treatment of malaria disease with chemotherapeutic agents remains the cornerstone of patient management and will likely remain so for the foreseeable future. But, albeit much can be done with existing drugs if properly used, there is an urgent need for new drugs with mode of actions different from existing ones. The reason is that resistance to antimalarials is emerging and spreading, and there are few effective drugs in the pipeline[1].

Drug research should be led by, and adjusted to, the needs of malaria treatment in the field so that research products can meet and satisfy the diverse practical requirements of case management in different situations. Needs for malaria drugs include life-saving therapy and drugs for broad use for uncomplicated malaria – many of the drugs for current use are restrained by resistance, cost or toxicity in target groups. It is equally important that the cost-benefit and the acceptability of new interventions receive careful evaluation at an early stage of development.

There are short-term and long-term research objectives. A reasonable objective in the short term would be to optimize the use and effective life-span of available drugs: exploring drug combinations would be critical to improve dosing regimens and minimize the development of resistance. Longer term, though unavoidable, objectives are the identification of chemotherapeutic targets and the discovery of new leads, as well as a better understanding of the mechanisms of drug action and resistance.

The needs

Treatment of severe malaria

Mortality due to cerebral malaria is still high; case-fatality rates with standard treatment range 12-25% depending on prevailing standard of care, even in areas of full quinine sensitivity[2]. Quinine is safe but its efficacy is fading in Thailand[3].

The needs in connection with the management of severe malaria are three fold: developing new antimalarials unrelated to quinine to overcome resistance; assess alternative approaches to prevent progression to severe malaria; better understanding of the pathogenesis of severe malaria to identify adjunct treatment.

Artemisinin derivatives (artemether, arteether, artesunate, dihydroartemisinin) show no cross-resistance with other antimalarials and have rapid activity[4, 5]. With the exception of artesunate they are all insoluble in water and therefore cannot be administered intravenously. At present, no conclusive comparative study is available to enable to opt for one derivative or an-

other. The use of these drugs has traditionally been limited to China and areas of Southeast Asia, but has more recently spread to the African continent. For most products though, the available pre-clinical and clinical data do not satisfy stringent regulatory requirements for international marketing authorization.

It is worthwhile noting that so far, studies have failed to show a clear clinical advantage in terms of mortality and sequelae of artemisinin derivatives over quinine[6-10]. This finding highlights the need for alternative approaches to prevent the occurrence of the serious complications of falciparum malaria. Ideally, prompt diagnosis and treatment preferably of uncomplicated cases would settle the problem, but in practice very often patients – mostly children – are already comatous when they reach the hospital. That is why it has been suggested that control strategies should be directed against the disease rather than killing the parasite since children in endemic areas develop immunity to the serious complications of malaria several years before their parasitaemias start to fall[11]. Better understanding of mechanisms involved in the pathogenesis of severe malaria (cytokines, cytoadherence and rosetting) is therefore needed[12, 13].

Treatment of uncomplicated malaria episodes

The therapeutic armamentarium for treating malaria is limited, and drug cross-resistance is facilitated by the fact that most of the available compounds belong to a restricted collection of chemical structures. Chloroquine has been for decades the mainstay of malaria treatment and prevention, but resistance to this drug is nowadays widely spread[14, 15]. Second-line treatment in Africa is pyrimethamine/sulfadrug combinations, but resistance is emerging after a relatively short time from introduction. Toxicity (particularly severe cutaneous adverse events) may also limit its use. In fact, the blessing and the drawback of the in-use antifolates has been their long half-lives, which can generate resistance and be responsible for drug adverse reactions[16, 17]. In certain areas of Southeast Asia, multidrug resistant parasites have emerged and there is evidence for reduced efficacy of almost all known antimalarials. Resistance is already established to mefloquine and halofantrine in Southeast Asia, particularly Thailand[14, 15, 18-20] and sporadically elsewhere[21, 22]. The use of halofantrine is limited by cross-resistance with mefloquine and possible cardiac toxicity[23].

Although artemisinin derivatives rapidly reduce parasitemia, parasite recrudescence occurs frequently unless given for 5 days or longer or used in combination with mefloquine[24-28]. But concerns are expressed over the use of oral formulations of artemisinin derivatives for the treatment of uncomplicated malaria in areas where other first-line medications could be used: unregulated use of these drugs may exert drug pressure that can lead to resistance; moreover, the effect of repeat dosing is not known yet and might lead to cumulative (neuro- or cardio-) toxicity so far observed only in experimental animals[29, 30]. The long-term implications of their use need to be assessed, and new derivatives with better disposition, oral bioavailability and toxicological profile need to be sought.

Thus, there appear to be two sets of priorities in connection with these patterns of resistance: one is for a first-line, safe, oral, preferably cheap medication for broad use to replace chloroquine, particularly for Africa. In addition, drugs effective in multidrug-resistant malaria are badly needed in limited areas of the world nowadays. For the time being the numbers of people in need of such drugs are comparably small and relatively high priced medications can be afforded. But the world may also face the appalling prospect of multidrug resistant strains spreading to high transmission areas of Africa and this would be a crisis of immense proportion[21, 22].

Waiting for new drugs with novel mechanisms of action to become available, a rationale assessment of combination chemotherapy regimens should be carefully done in order to prevent or delay parasite resistance[31].

The reduced efficacy of drugs against blood stages makes it increasingly important to develop causal prophylactic or radical curative agents, thus preventing blood stages from emerging and causing clinical diseases.

Perspectives

The previous section has highlighted the compelling need for novel antimalarials. Chemotherapeutic targets and new lead compounds have been identified, however, the drug development pipeline is drying off. Interesting though they are, the new avenues in research appear to have little impact on the ultimate availability of new drugs on the pharmacy shelves. Moreover, the costs and risks of drug development, particularly in the face of the perceived limited profits and the long payback period of drugs for the developing world, are seen by the pharmaceutical industry as disincentives for antimalarial drug development.

New drugs under development[32]

New injectable artemisinin derivatives are currently being developed: arteether, closely related to artemether, is water-insoluble and such injected intramuscularly, is in Phase III clinical development; sodium artelinate, water-soluble, is in preclinical development. Also existing artemisinin derivatives are receiving further study, including potential combinations with other antimalarial drugs[24-28], and alternative formulations. Studies are being conducted to assess whether the availability and use at the peripheral level of the health system of artesunate suppositories for patients unable to swallow "en route" to the hospital can prevent severe malaria or improve its outcome.

Atovaquone (hydroxynaphthoquinone), is a new antimalarial with a broad spectrum of activity to opportunistic pathogens (*T. gondii, P. carinii*). It shows high intrinsic activity against erythrocytic stages of *P. falciparum in vitro* and also primary liver stages. As an inhibitor of electron transport, it has a novel mode of action and is not cross resistant with other antimalarials. In contrast to previous members of the naphthoquinone series, it is metabolically stable with an elimination half-life of approximately seventy hours. However, resistant parasites emerge readily after single-agent treatment with atovaquone in approximately 30% of cases; atovaquone is therefore now combined with proguanil to prevent or delay emergence of resistance, and is currently in advanced phases of international clinical development for the treatment of uncomplicated *P. falciparum* malaria. The combination appear to be very effective in the various settings where it was tested[33, 34].

The combination of chlorproguanil and dapsone proved to have the best therapeutic index among available drugs investigated *in vitro* to assess the activity and toxicity of antifolate drug combination[35]. This combination was then chosen for clinical trials, also based on preliminary clinical results and good track of safe use of the two individual drugs[36]: the results of a Phase II trial in Kenya show encouraging results with a three-day regimen with chlorproguanil/dapsone vs pyrimethamine/sulfadoxine (P. Winstanley, personal communication). The rationale for using alternative antifolates with shorter residence in the organism is two fold. First, due to their short half life they are believed to exert lesser selective pressure on the parasites and to be less prone to toxicity. Second, pyrimethamine and sulphonamide resistance are unlinked in *P. falciparum*[37, 38]. But this approach, which may be valid in areas of no or just emerging antifolate resistance, may not be enough in areas of Southeast Asia with high levels of resistance to both DHFR and dihydropteroate synthase (DHPS) inhibitors. There, newer drugs are required, such as the triazine WR99210, administered as the prodrug PS-15 (or WR250417)[39].

There are also other drugs under development that share common chemical structures with in-use drugs. Despite common chemical features, Mannich bases (eg. quinoline-type: amodiaquine, amopyroquine; acridine-type: pyronaridine, pyracrine) differ substantially from chloroquine in their activities and in the rate and stability of resistance. The development of amopyroquine was abandoned because of high cost of production, minimal advantage over amodiaquine, and poor oral bioavailability. Amodiaquine -which has long been underutilised after reports of severe and fatal haematological toxicity in prophylaxis- has still a place in the treatment of uncomplicated malaria as it is comparatively more active and no more

toxic than chloroquine[42]. Pyronaridine stands out as the more active molecule of the whole lot. It has been used clinically in China since the 1970s and is now marketed in that country, but is not widely available and has been only used experimentally outside China. Two recent studies in Cameroon and Thailand have shown the drug is effective against chloroquine-resistant parasites and retains activity in areas of multidrug resistance[43, 44, 45]. Pyronaridine is a potentially valid addition to the therapeutic armamentarium for the treatment of malaria. Nevertheless, a larger accessibility of the drug is conditional to substantial investments in the studies required to meet international standards for registration outside China in light of the possible life-span of the compound. Also, the current price makes is by far too expensive for extensive use: dosing regimes need to be optimised and clinical studies with more suited oral formulations are underway[30].

Another Chinese synthetic compound benflumetol, a fluoromethanol, is currently in Phase III clinical development for concurrent use with artemether.

Primaquine replacements under investigation are WR238 605 and CDRI 80/53. WR238 605, an 8-aminoquinoline compound which was originally designed as a primaquine-replacement for prevention of relapsing malaria, shows promising activity for prevention and treatment of *falciparum* malaria. It is currently in phase II clinical testing. Its potential advantages over primaquine are: a longer half-life, a better therapeutic index, a greater blood schizontocidal activity in addition to its activity as a tissue schizontocidal[46]. It is hoped that this drug can be used for causal prophylaxis and as single-dose radical curative for *P. vivax*. CDRI 80/53 has comparable activity with primaquine but is claimed to have lesser haemolytic potential.

Some alternate approaches

As pointed out before, several other factors beyond "parasite susceptibility" contribute to malaria mortality, hence the need for adjuvant treatment and "antidisease" therapy to prevent progression to severe malaria. Antagonists of Tumor Necrosis Factor (TNF) are being developed and tested by several pharmaceutical companies to combat septic shock and autoimmune diseases and some of them have been tested clinically in severe malaria, but the results are not encouraging so far (B. Greenwood, personal communication). Preliminary data indicate pentoxyfillin may be a valid adjunction to standard quinine treatment[47, 48]. Putative receptors and ligands on the surface of infected cells which are involved in cytoadherence and in rosetting have been identified. These could lead to novel interventions for the management of severe malaria. It is hoped that ongoing research can lead to drugs that, combined with anti-parasite therapy, would reduce malaria-associated morbidity and mortality.

Restoring susceptibility to antimalarials to which parasites have become resistant could also be a valuable solution. Various drugs, including calcium-channel inhibitors (e.g. verapamil) and tricyclic compounds (e.g. desipramine) that are known to reverse the mdr phenotype in human cells have been shown to "reverse" or "modify" chloroquine resistance *in vitro*[49, 50]. Others, like penfluridol, modulate resistance to mefloquine and halofantrine. Unfortunately, no compound has yet proved effective *in vivo* and there is some concern for potential host cell toxicity[51]. The availability of such resistance modifiers or chemosensitizing compounds would have great practical repercussions by restoring the effectiveness of present first-line antimalarial drugs in areas where parasites are no longer susceptible. This would be particularly desirable for chloroquine, which was shown to be valuable also in inhibiting cytokine production[52].

Long-term goals: drug discovery

Achieving short-term objectives by "getting extra-mileage" from existing drugs or their combinations can momentarily ease the struggle for tools to control malaria, but will only partly offset the trend to further expansion of resistance. In fact, the current armamentarium of drugs against malaria is limited, and this appears to be one of the factors which have contributed

to the development of drug resistance. Therefore, long-term objectives of research must focus on the discovery of compounds with new structures acting on new chemotherapeutic targets.

More research needs to be done in order to identify and validate targets, and to use them for testing compound activity. However we cannot expect all targets to be suited; a chemotherapeutic target must be an essential feature of the parasite life-cycle (involved in a rate limiting biochemical process with no alternative pathways which circumvent the target), and either parasite-specific (e.g. food vacuole functions, the mitochondrion/plastid) or differ significantly from any analogous process in the host (e.g. DHFR, signal transduction). A number of putative chemotherapeutic targets have been identified for malaria.

Examples of validated targets already amenable to compound screening are: proteinase inhibition in the plasmodium food vacuole (plasmepsin I, II, and cystein proteinase) and inhibition of folate metabolism (DHFR, DHPS). Inhibitors of phospholipid metabolism are being tested. Other drug targets are at earlier stages of investigation or have not yet led to the discovery of new leads. Chemotherapeutic targets for malaria are reviewed in Vial & Olliaro, 1994[53].

Ultimately, the main motor for change will have to come from technology, and already recent discoveries and developments open new avenues of research for drug discovery. There are already several examples of new technologies which can be increase our capability to discover new compounds: recent advances in genetic engineering now allow to identify and confirm targets; the malaria genome is being sequenced, and this has the potential for leading to new targets identification; huge numbers of compounds can now be tested by *robotic high throughput screening* is possible against selected enzyme targets. The capability to generate new structures is also now tremendously improved by new techniques such as *combinatorial chemistry*. This consists of a systematic assembly of molecular "building blocks" in multiple combinations and generates collections of compounds named *combinatorial libraries*. These molecules can be either biological (DNA/RNA, peptides/proteins) or chemical (peptides, polymers or peptoids, and now also organic). In essence, the rationale for combinatorial chemistry is twofold. Firstly, many believe that our difficulty in discovering novel effective molecules stems from the limited numbers of chemical structures explored so far - combinatorial chemistry aims at generating chemical *diversity*. Secondly, compound screening and lead optimisation by medicinal chemistry work has traditionally been a bottleneck in drug discovery; now many would hope that chemical libraries may lead to a drug with minimal medicinal chemistry. By coupling combinatorial chemistry and robotic high throughput screening it is now hoped that huge numbers of compounds in produced in minimal quantities can be screened in very limited times. This new technique is now being adopted by several pharmaceutical companies as an alternative to more classical approaches to drug discovery.

But, since parasites appear to invariably get round any human intervention, research should also focus on understanding the mechanisms of resistance, if we are ever going to keep pace with the development of resistance.

The research and development (R&D) process for drugs

The rate at which such new drug entities will become available for clinical use depends on the available resources. Over the past ten years, there has been a significant cut in funds allocated to drug discovery in the drug industry, and the number of new chemical entities has dropped substantially. This affects primarily low-priority areas for the pharmaceutical industry such as tropical diseases. The burden for development is therefore on the public sector, however, governmental efforts also are being reduced significantly.

New chemical entities discovered through the process mentioned above cannot be expected to become drugs available on the market soon. The drug discovery and development process is lengthy and expensive. The timescale now averages 12 years, and can fail at any stage. Full R&D costs for each new chemical entity on the market av-

erage US$ 395 million -costs inclusive of projects prematurely terminated; a commonly accepted figure is that 1 in 10,000 molecules studied is ultimately put on the market. Review of dossiers by regulatory authorities also needs time: the review process by the Food and Drug Administration in the United States of America takes on average 32 months[54].

Conclusions

Much is being done for malaria but this is obviously not enough. The dominating theme in antimalarial use and research is resistance, which is the key factor in determining the duration of a drug effective lifespan.

The efficacy and safety of existing antimalarials could be "protected" if we could use them better and combine them rationally. But these would represent only momentary solutions, and there are obvious difficulties in regulating the use of antimalarials when these are freely available in the market-place.

New drugs need to differ in their chemical structures from existing ones and to target different processes of the parasite, and as such artemisinin derivatives and atovaquone/proguanil represent recent breakthroughs. But will they be accessible and used properly? How long will their efficacy last? When can we expect other drugs to become available? The duration, costs and risk of the drug R&D process work against having new antimalarials available soon. There are no many antimalarial compounds in the pipeline, and although there appear to be new interesting prospects, these are most often at very preliminary stages, and one should not forget that the risk of failure is highest in the early phases of the drug development process. Although several new targets have been identified, we cannot expect all of them to eventually prove essential and specific. The ultimate need is for basic research to discover new targets and lead compounds and to elucidate the mechanisms of resistance.

Finally, people should realise that development and implementation of antimalarial drugs needs a concerted venture, and that efforts are doomed to failure if a partnership is not created between public and private sector.

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